



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**
(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2017/08/15 (87) Date publication PCT/PCT Publication Date: 2018/02/22 (85) Entrée phase nationale/National Entry: 2019/02/14 (86) N° demande PCT/PCT Application No.: US 2017/046930 (87) N° publication PCT/PCT Publication No.: 2018/035116 (30) Priorité/Priority: 2016/08/16 (US62/375,689)	(51) Cl.Int./Int.Cl. <i>C07K 1/34</i> (2006.01) (71) Demandeur/Applicant: GENZYME CORPORATION, US (72) Inventeurs/Inventors: VETTER, TARL, US; COOLBAUGH, MICHAEL, US; WARIKOO, VEENA, US; KONSTANTINOV, KONSTANTIN, US (74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : PROCEDES DE TRAITEMENT D'UN FLUIDE COMPRENANT UNE PROTEINE THERAPEUTIQUE RECOMBINANTE ET LEUR UTILISATION
(54) Title: METHODS OF PROCESSING A FLUID INCLUDING A RECOMBINANT THERAPEUTIC PROTEIN AND USE THEREOF

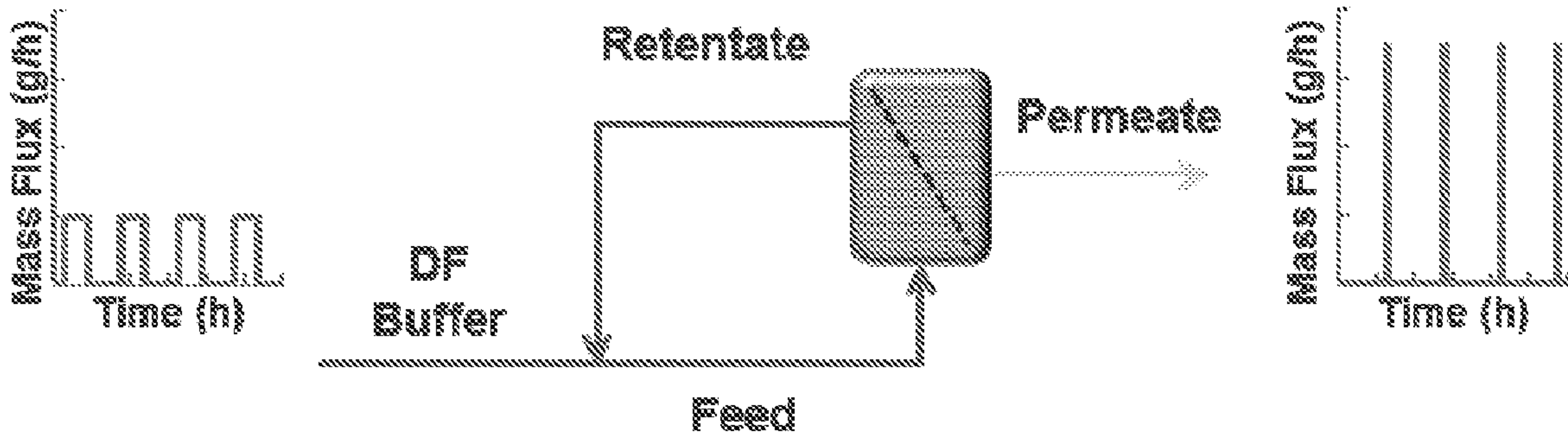


FIG. 6

(57) **Abrégé/Abstract:**
Provided herein are methods of processing a fluid that include the use of one or both of a circuit system including a tangential flow filtration (TFF) unit and a circuit system including a tangential flow virus filtration (TFVF) unit. Also provided are integrated and continuous processes for manufacturing a therapeutic protein drug substance that include a step of processing a fluid including the recombinant therapeutic protein using any of the methods provided herein.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
22 February 2018 (22.02.2018)



(10) International Publication Number
WO 2018/035116 A1

(51) International Patent Classification:
C07K 1/34 (2006.01)

(21) International Application Number:
PCT/US2017/046930

(22) International Filing Date:
15 August 2017 (15.08.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/375,689 16 August 2016 (16.08.2016) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,

HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))

(54) Title: METHODS OF PROCESSING A FLUID INCLUDING A RECOMBINANT THERAPEUTIC PROTEIN AND USE THEREOF

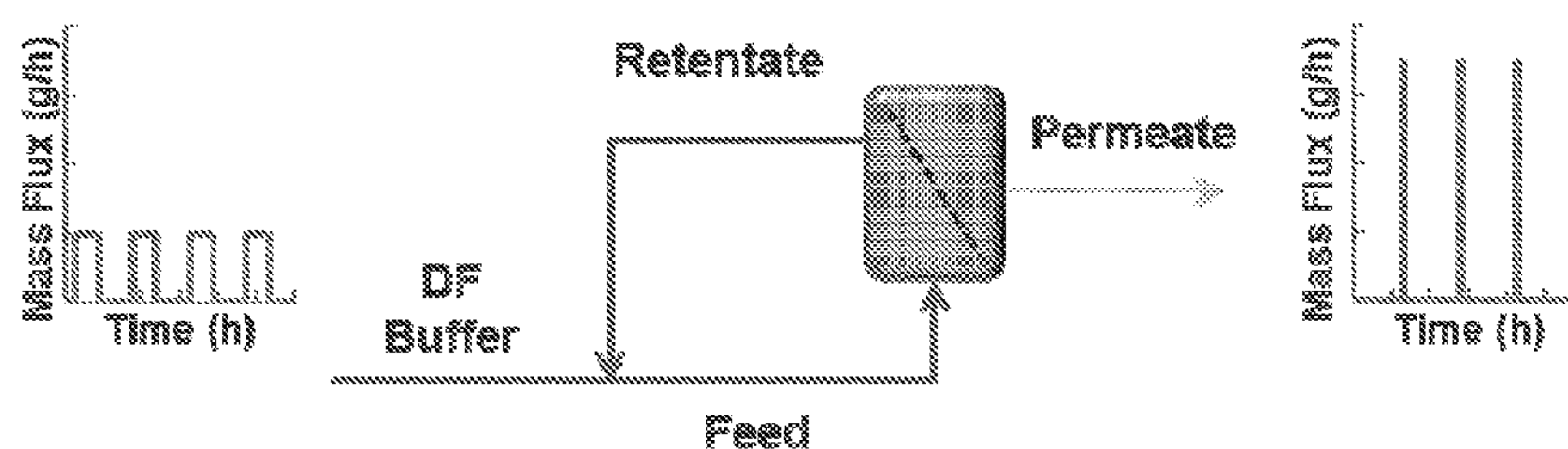


FIG. 6

(57) Abstract: Provided herein are methods of processing a fluid that include the use of one or both of a circuit system including a tangential flow filtration (TFF) unit and a circuit system including a tangential flow virus filtration (TFVF) unit. Also provided are integrated and continuous processes for manufacturing a therapeutic protein drug substance that include a step of processing a fluid including the recombinant therapeutic protein using any of the methods provided herein.

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DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

**METHODS OF PROCESSING A FLUID INCLUDING A
RECOMBINANT THERAPEUTIC PROTEIN AND USE THEREOF**

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Patent Application Serial No. 62/375,689, filed August 16, 2016; the entire contents of which are herein incorporated by reference.

TECHNICAL FIELD

10 This invention relates to methods of biotechnology and the biomanufacturing of recombinant proteins.

BACKGROUND

15 Mammalian cells containing a nucleic acid that encodes a recombinant protein are often used to produce therapeutically or commercially important proteins. In the current environment of diverse product pipelines, biotechnology companies are increasingly driven to develop innovative solutions for highly flexible and cost-effective manufacturing of therapeutic protein drug substances.

SUMMARY

20 The present invention is based, at least in part, on the discovery that circuit systems including a tangential flow filtration (TFF) unit or a tangential flow virus filtration (TFVF) unit can be used to continuously perform the unit operation of ultrafiltration/diafiltration and virus filtration, respectively. In view of this discovery, provided herein are methods of processing a fluid that include the use of one or both
25 of a circuit system including a tangential flow filtration (TFF) unit and a circuit system including a tangential flow virus filtration (TFVF) unit. Also provided are integrated and continuous processes for manufacturing a therapeutic protein drug substance that include a step of processing a fluid including the recombinant therapeutic protein using any of the methods provided herein.

30 Provided herein are methods of processing a fluid including a recombinant therapeutic protein that include: (a) providing a circuit system including (i) a tangential flow filtration (TFF) unit having first and second inlets, and (ii) a conduit in

fluid communication between the first and second inlets, including at least one port for flowing fluid into or out of, or both, of the system, where the system is configured such that fluid can be flowed through the conduit and the TFF unit, and filtrate not including the recombinant therapeutic protein can be collected from the TFF unit; (b) continuously flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port, and discarding filtrate not including the recombinant therapeutic protein for a first period of time; (c) continuously flowing a diafiltration medium into the circuit system through one of the at least one port, and discarding filtrate not including the recombinant therapeutic protein for a second period of time; and (d) collecting a fluid including the recombinant therapeutic protein that is present in the circuit system after the first and second time periods through one of the at least one port. In some embodiments of these methods, step (b) includes continuously flowing the fluid into the circuit system at a rate of about 0.1 mL/minute to about 100 L/minute (e.g., about 0.5 mL/minute to about 200 mL/minute). In some embodiments of these methods of these methods, the first period of time is about 0.1 minute to about 12 hours (e.g., about 10 minutes to about 4 hours). In some embodiments of these methods, the flowing of the fluid into the circuit system in step (b) occurs unidirectionally. In some embodiments of these methods, step (c) includes continuously flowing the diafiltration medium into the circuit system at a rate of about 0.1 mL/minute to about 100 L/minute (e.g., about 0.5 mL/minute to about 200 mL/minute). In some embodiments of these methods, the second period of time is about 5 minutes to about 12 hours (e.g., about 10 minutes to about 4 hours). In some embodiments of these methods, the flowing of the diafiltration medium into the circuit system in step (c) occurs unidirectionally. In some embodiments of these methods, the conduit has an inner diameter of about 5 mm to about 5 cm (e.g., about 10 mm to about 3 cm). In some embodiments of these methods, the TFF unit comprises one or more tangential filter(s) having a surface area of about 0.1 cm² to about 50 cm² (e.g., about 0.5 cm² to about 20 cm²). In some embodiments of these methods, the circuit system further includes a pump (e.g., a peristaltic pump) disposed in the conduit.

Some embodiments of these methods further include: (e) providing a second circuit system including (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and

second inlets of the TFVF unit, including at least one port for flowing fluid into or out of, or both, of the second system, where the second system is configured such that fluid can be flowed through the conduit and the TFVF unit, and filtrate including the recombinant therapeutic protein can be collected from the TFVF unit; and (f) flowing
5 the collected fluid of step (d) into the second system using a connecting conduit in fluid communication between a port in the system and a port in the second system, and collecting filtrate including the recombinant therapeutic protein from the TFVF unit for a third period of time. In some embodiments of these methods, step (f) includes flowing the fluid into the second system at a rate of about 0.1 mL/minute to
10 about 100 L/minute (e.g., about 0.5 mL/minute to about 200 mL/minute). In some embodiments of these methods, the third period of time is about 0.1 minute to about 12 hours (e.g., about 10 minutes to about 2 hours). In some embodiments of these methods, the flowing of the fluid into the second system in step (f) occurs unidirectionally. In some embodiments of these methods, the conduit in fluid
15 communication between the first and second inlets of the TFVF unit has an inner diameter of about 5 mm to about 5 cm (e.g., about 10 mm to about 3 cm). In some embodiments of these methods, the TFVF unit includes one or more tangential virus filter(s) having a surface area of about 0.1 cm² to about 100 m² (e.g., about 0.5 cm² to about 200 cm²). In some embodiments of these methods, the connecting conduit has
20 an inner diameter of about 5 mm to about 10 cm (e.g., about 10 mm to about 5 cm). In some embodiments of these methods, the second system further includes a pump (e.g., a peristaltic pump) disposed in the conduit in fluid communication between the first and second inlets of the TFVF unit.

Also provided are methods for processing a fluid including a recombinant
25 therapeutic protein that include: (a) providing a circuit system including (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets of the TFVF unit, including at least one port for flowing fluid into or out of, or both, of the system, where the system is configured such that fluid can be flowed through the conduit and
30 the TFVF unit, and filtrate including the recombinant therapeutic protein can be collected from the TFVF unit; (b) continuously flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port, and collecting filtrate including the recombinant therapeutic protein from the

TFVF unit for a period of time. In some embodiments of these methods, step (b) includes continuously flowing the fluid into the system at a rate of about 0.1 mL/minute to about 100 L/minute (e.g., about 0.5 mL/minute to about 200 mL/minute). In some embodiments of these methods, the period of time is about 5 minutes to about 12 hours (e.g., about 10 minutes to about 3 hours). In some embodiments of these methods, the flowing of the fluid into the system in step (b) occurs unidirectionally. In some embodiments of these methods, the conduit has an inner diameter of about 5 mm to about 5 cm (e.g., about 10 mm to about 3 cm). In some embodiments of these methods, the TFVF unit includes one or more tangential virus filter(s) having a surface area of about 0.1 cm² to about 100 m² (e.g., about 0.5 cm² to about 200 cm²). In some embodiments of these methods, the system further includes a pump (e.g., a peristaltic pump) disposed in the conduit.

Also provided are integrated and continuous processes for manufacturing a therapeutic protein drug substance that include: (a) providing a liquid culture medium including a recombinant therapeutic protein that is substantially free of cells, where the liquid culture medium is fed into a first multi-column chromatography system (MCCS1); (b) capturing said recombinant therapeutic protein in the liquid culture medium using the MCCS1, where the eluate of the MCCS1 including the recombinant therapeutic protein is continuously fed into a second multi-column chromatography system (MCCS2); (c) purifying and polishing the recombinant therapeutic protein using the MCCS2, wherein the eluate from the MCCS2 is a fluid including the recombinant therapeutic protein; (d) processing the fluid including the recombinant therapeutic protein using a method of any methods described herein; where the process is integrated and runs continuously from said liquid culture medium to the collected fluid or filtrate including the recombinant therapeutic protein, where the collected fluid or filtrate is the therapeutic protein drug substance. In some embodiments of these processes, the MCCS1 performs at least two different unit operations. In some embodiments of these processes, the MCCS1 performs the unit operations of capturing the recombinant therapeutic protein and inactivating viruses. In some embodiments of these processes, the use of the MCCS1 or the MCCS2, or both, involves column switching. In some embodiments of these processes, the MCCS1 and/or MCCS2 utilizes at least two chromatography columns. In some embodiments of these processes, the MCCS1 and/or MCCS2 utilizes at least two

chromatographic membranes. In some embodiments of these processes, the MCCS1 and/or MCCS2 utilizes at least one chromatography column and at least one chromatographic membrane. In some embodiments of these processes, the MCCS1 is a first periodic counter current chromatography system (PCCS1). In some
5 embodiments of these processes, the PCCS1 includes a four-column PCCS. In some embodiments of these processes, three of the four columns in the four-column PCCS perform the unit operation of capturing the recombinant therapeutic protein from the liquid culture medium. In some embodiments of these processes, the capturing is performed using affinity chromatography, cation exchange chromatography, anion
10 exchange chromatography, or molecular sieve chromatography. In some embodiments of these processes, the affinity chromatography is performed with a capture mechanism selected from the group of: protein A-binding capture mechanism, substrate-binding capture mechanism, antibody- or antibody fragment-binding capture mechanism, aptamer-binding capture mechanism, and cofactor-binding capture
15 mechanism. In some embodiments of these processes, the affinity chromatography is performed with a protein A-binding capture mechanism, and the recombinant therapeutic protein is an antibody or an antibody fragment. In some embodiments of these processes, the eluate containing the recombinant therapeutic protein from three of the four columns in the four-column PCCS is fed into the fourth column of the
20 four-column PCCS. In some embodiments of these processes, the fourth column of the four-column PCCS performs the unit operation of inactivating viruses by holding the eluate containing recombinant therapeutic protein at a low pH for viral inactivation. In some embodiments of these processes, the fourth column of the four-column PCCS holds the eluate containing the recombinant protein at a low pH for
25 viral inactivation for a period of about 10 minutes to about 1.5 hours.

In some embodiments of these processes, the MCCS2 is a second periodic counter current chromatography system (PCCS2). Some embodiments of these processes further include adjusting the pH of the eluate from the fourth column of the four-column PCCS using an in-line buffer adjustment reservoir before the eluate from
30 the fourth column of the four-column PCCS is fed into the PCCS2. In some embodiments of these processes, the PCCS2 includes three chromatography columns and a chromatographic membrane. In some embodiments of these processes, the three chromatographic columns in the PCCS2 perform the unit operation of purifying

the recombinant therapeutic protein from the eluate of the PCCS1 through cation or anion exchange chromatography. In some embodiments of these processes, the eluate from the three chromatography columns in the PCCS2 is fed into the chromatographic membrane in the PCCS2. In some embodiments of these processes, the chromatographic membrane in the PCCS2 performs the unit operation of polishing the recombinant therapeutic protein present in the eluate from the three chromatographic columns in the PCCS2 through cation or anion exchange chromatography. In some embodiments of these processes, the chromatographic membrane in the PCCS2 performs the unit operation of polishing through cation exchange chromatography. In some embodiments of these processes, the flow through and wash of the chromatographic membrane includes the recombinant therapeutic protein. Some embodiments of these processes further include adjusting the ionic concentration of the eluate from the three columns in the PCCS2 using in-line buffer adjustment before the eluate from the three columns in the PCCS2 is fed into the chromatographic membrane in the PCCS2. Some embodiments of these processes further include the use of a break tank between the PCCS1 and the PCCS2. Some embodiments of these processes further include filtering the eluate from the PCCS1 before it is fed into the PCCS2. Some embodiments of these processes further include formulating the therapeutic protein drug substance into a pharmaceutical composition. In some embodiments of any of methods or processes described herein, the recombinant therapeutic protein is an antibody or antibody fragment, an enzyme, an engineered protein, or an immunogenic protein or protein fragment.

As used herein, the word “a” before a noun represents one or more of the particular noun. For example, the phrase “a mammalian cell” represents “one or more mammalian cells.”

The terms “tangential flow filtration unit” or “TFF unit” are art-known and mean a device that includes at least one housing (such as a cylinder) and at least one cross-flow (tangential) filter positioned in the housing such that a large portion of the filter’s surface is positioned parallel to the flow of a fluid (e.g., a cell culture) through the unit. TFF units are well-known in the art and are commercially available. The housing can include a first inlet/outlet and a second inlet/outlet positioned, e.g., to allow fluid to pass through the first inlet/outlet, cross the at least one cross-flow filter, and through the second inlet/outlet. In some examples, a circuit system can include

multiple TFF units, e.g., connected in series and/or in parallel. For example, a circuit system that includes two or more TFF units can include fluid conduits fluidly connecting neighboring pairs of TFF units in the system. In other examples, a circuit system can include two or more sets of two or more TFF units fluidly connected by fluid conduits. Any of the TFF units described herein or known in the art are capable of receiving fluid in a first flow direction and a second flow direction.

The terms “tangential flow virus filtration unit” or “TFVF unit” are art-known and mean a device that includes at least one housing (such as a cylinder) and at least one cross-flow (tangential) virus filter positioned in the housing such that a large portion of the virus filter’s surface is positioned parallel to the flow of a fluid (e.g., a cell culture) through the unit. The housing can include a first inlet/outlet and a second inlet/outlet positioned, e.g., to allow fluid to pass through the first inlet/outlet, cross the at least one cross-flow virus filter, and through the second inlet/outlet. In some examples, a circuit system can include multiple TFVF units, e.g., connected in series and/or in parallel. For example, a circuit system that includes two or more TFVF units can include fluid conduits fluidly connecting neighboring pairs of TFVF units in the system. In other examples, a circuit system can include two or more sets of two or more TFVF units fluidly connected by fluid conduits. Any of the TFVF units described herein or known in the art are capable of receiving fluid in a first flow direction and a second flow direction.

The term “cross-flow filter” or “tangential filter” is art known and means a filter that designed such that it can be positioned in a TFF or a TFVF unit such that a large portion of the filter’s surface is parallel to the flow (e.g., first and second flow direction) of a fluid (e.g., a fluid including a recombinant therapeutic protein). For example, a cross-flow filter can have any shape that allows for tangential flow filtration, e.g., a tubular or rectangular shape. Particularly useful cross-flow filters are designed to result in a low amount of fluid turbulence or sheer stress in the fluid (e.g., cell culture) when the fluid is flowed (e.g., unidirectionally flowed to bidirectionally flowed) across the surface of the cross-flow filter. Cross-flow filters are commercially available, e.g., from Sartorius, MembraPure, Millipore, and Pall Corporation.

The term “low turbulence pump” or “LTP” is art-known and means a device that can move a fluid (e.g., a fluid including a recombinant therapeutic protein) within

a system or circuit in a single direction (e.g., a first or second flow direction) or reversibly flowing a fluid (e.g., a fluid including a recombinant therapeutic protein) in two directions (a first and second flow direction) within the system without inducing a substantial amount of sheer stress or fluid turbulence in the fluid (e.g., a fluid including a recombinant therapeutic protein). When a LTP is used to flow a fluid (e.g., a fluid including a recombinant therapeutic protein) in alternating first and second flow directions, the second flow direction is approximately opposite to that of the first flow direction. An example of a LTP is a peristaltic pump. Other examples of LTPs are known in the art.

The term “mammalian cell” means any cell from or derived from any mammal (e.g., a human, a hamster, a mouse, a green monkey, a rat, a pig, a cow, or a rabbit). For example, a mammalian cell can be an immortalized cell. In some embodiments, the mammalian cell is a differentiated cell. In some embodiments, the mammalian cell is an undifferentiated cell. Non-limiting examples of mammalian cells are described herein. Additional examples of mammalian cells are known in the art.

The term “substantially free” means a composition (e.g., a liquid culture medium) that is at least or about 90% free (e.g., at least or about 95%, 96%, 97%, 98%, or at least or about 99% free, or about 100% free) of a specified substance (e.g., a mammalian cell).

The term “0.5x volume” means about 50% of the volume. The term “0.6x volume” means about 60% of the volume. Likewise, 0.7x, 0.8x, 0.9x, and 1.0x means about 70%, 80%, 90%, or 100% of the volume, respectively.

The term “culturing” or “cell culturing” means the maintenance or proliferation of a mammalian cell under a controlled set of physical conditions.

The term “culture of mammalian cells” means a liquid culture medium containing a plurality of mammalian cells that is maintained or proliferated under a controlled set of physical conditions.

The term “liquid culture medium” means a fluid that contains sufficient nutrients to allow a cell (e.g., a mammalian cell) to grow or proliferate *in vitro*. For example, a liquid culture medium can contain one or more of: amino acids (e.g., 20 amino acids), a purine (e.g., hypoxanthine), a pyrimidine (e.g., thymidine), choline, inositol, thiamine, folic acid, biotin, calcium, niacinamide, pyridoxine, riboflavin, thymidine, cyanocobalamin, pyruvate, lipoic acid, magnesium, glucose, sodium,

potassium, iron, copper, zinc, and sodium bicarbonate. In some embodiments, a liquid culture medium can contain serum from a mammal. In some embodiments, a liquid culture medium does not contain serum or another extract from a mammal (a defined liquid culture medium). In some embodiments, a liquid culture medium can contain trace metals, a mammalian growth hormone, and/or a mammalian growth factor. Another example of liquid culture medium is minimal medium (e.g., a medium containing only inorganic salts, a carbon source, and water). Non-limiting examples of liquid culture medium are described herein. Additional examples of liquid culture medium are known in the art and are commercially available. A liquid culture medium can contain any density of mammalian cells. For example, as used herein, a volume of liquid culture medium removed from a bioreactor can be substantially free of mammalian cells.

The term “animal-derived component free liquid culture medium” means a liquid culture medium that does not contain any components (e.g., proteins or serum) derived from a mammal.

The term “serum-free liquid culture medium” means a liquid culture medium that does not contain a mammalian serum.

The term “serum-containing liquid culture medium” means a liquid culture medium that contains a mammalian serum.

The term “chemically-defined liquid culture medium” is a term of art and means a liquid culture medium in which all of the chemical components are known. For example, a chemically-defined liquid culture medium does not contain fetal bovine serum, bovine serum albumin, or human serum albumin, as these preparations typically contain a complex mix of albumins and lipids.

The term “protein-free liquid culture medium” means a liquid culture medium that does not contain any protein (e.g., any detectable protein).

The term “agitation” means stirring or otherwise moving a portion of liquid culture medium in a bioreactor. This is performed in order to, e.g., increase the dissolved O₂ concentration in the liquid culture medium in a bioreactor. Agitation can be performed using any art known method, e.g., an instrument or propeller. Exemplary devices and methods that can be used to perform agitation of a portion of the liquid culture medium in a bioreactor are known in the art.

The term “therapeutic protein drug substance” means a recombinant protein (e.g., an immunoglobulin, protein fragment, engineered protein, or enzyme) that has been sufficiently purified or isolated from contaminating proteins, lipids, and nucleic acids (e.g., contaminating proteins, lipids, and nucleic acids present in a liquid culture medium or from a host cell (e.g., from a mammalian, yeast, or bacterial host cell)) and biological contaminants (e.g., viral and bacterial contaminants), and can be formulated into a pharmaceutical agent without any further substantial purification and/or decontamination step.

The term “integrated process” means a process which is performed using structural elements that function cooperatively to achieve a specific result (e.g., the generation of a therapeutic protein drug substance from a liquid culture medium).

The term “continuous process” means a process which continuously feeds fluid through at least a part of the system. For example, in any of the exemplary continuous biological manufacturing systems described herein, a liquid culture medium containing a recombinant therapeutic protein is continuously fed into the system while it is in operation and a therapeutic protein drug substance is fed out of the system. In another example, a continuous process is a process which continuously feeds a liquid culture medium containing a recombinant therapeutic protein from a bioreactor through a first MCCS. Another example of a continuous process is a process which continuously feeds a liquid culture medium containing a recombinant therapeutic protein from a bioreactor through a first and second MCCS. Additional examples include a process which continuously feeds a liquid culture medium containing a recombinant therapeutic protein through a first MCCS, a process that continuously feeds a liquid culture medium containing a recombinant therapeutic protein through a first and second MCCS, or a process that continuously feeds a fluid containing a recombinant therapeutic protein through a second MCCS.

The term “immunoglobulin” means a polypeptide containing an amino acid sequence of at least 15 amino acids (e.g., at least 20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids) of an immunoglobulin protein (e.g., a variable domain sequence, a framework sequence, or a constant domain sequence). The immunoglobulin may, for example, include at least 15 amino acids of a light chain immunoglobulin, e.g., at least 15 amino acids of a heavy chain immunoglobulin. The immunoglobulin may be an isolated antibody (e.g., an IgG, IgE, IgD, IgA, or IgM). The immunoglobulin may

be a subclass of IgG (e.g., IgG1, IgG2, IgG3, or IgG4). The immunoglobulin may be an antibody fragment, e.g., a Fab fragment, a F(ab')₂ fragment, or an scFv fragment. The immunoglobulin may also be a bi-specific antibody or a tri-specific antibody, or a dimer, trimer, or multimer antibody, or a diabody, an Affibody®, or a Nanobody®.

5 The immunoglobulin can also be an engineered protein containing at least one immunoglobulin domain (e.g., a fusion protein). Non-limiting examples of immunoglobulins are described herein and additional examples of immunoglobulins are known in the art.

The term “protein fragment” or “polypeptide fragment” means a portion of a polypeptide sequence that is at least or about 4 amino acids, at least or about 5 amino acids, at least or about 6 amino acids, at least or about 7 amino acids, at least or about 8 amino acids, at least or about 9 amino acids, at least or about 10 amino acids, at least or about 11 amino acids, at least or about 12 amino acids, at least or about 13 amino acids, at least or about 14 amino acids, at least or about 15 amino acids, at least or about 16 amino acids, at least or about 17 amino acids, at least or about 18 amino acids, at least or about 19 amino acids, or at least or about 20 amino acids in length, or more than 20 amino acids in length. A recombinant protein fragment can be produced using any of the processes described herein.

The term “engineered protein” means a polypeptide that is not naturally encoded by an endogenous nucleic acid present within an organism (e.g., a mammal). Examples of engineered proteins include enzymes (e.g., with one or more amino acid substitutions, deletions, insertions, or additions that result in an increase in stability and/or catalytic activity of the engineered enzyme), fusion proteins, antibodies (e.g., divalent antibodies, trivalent antibodies, or a diabody), and antigen-binding proteins that contain at least one recombinant scaffolding sequence.

The term “multi-column chromatography system” or “MCCS” means a system of a total of two or more interconnected or switching chromatography columns and/or chromatographic membranes. A non-limiting example of a multi-column chromatography system is a periodic counter current chromatography system (PCC) containing a total of two or more interconnected or switching chromatography columns and/or chromatographic membranes. Additional examples of multi-column chromatography systems are described herein and are known in the art.

The term “capturing” means a step performed to partially purify or isolate (e.g., at least or about 5%, e.g., at least or about 10%, 15%, 20%, 25%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or at least or about 95% pure by weight), concentrate, and stabilize a recombinant therapeutic protein from one or more other components present in a liquid culture medium or a diluted liquid culture medium (e.g., culture medium proteins or one or more other components (e.g., DNA, RNA, or other proteins) present in or secreted from a mammalian cell). Typically, capturing is performed using a resin that binds a recombinant therapeutic protein (e.g., through the use of affinity chromatography). Non-limiting methods for capturing a recombinant therapeutic protein from a liquid culture medium or diluted liquid culture medium are described herein and others are known in the art. A recombinant therapeutic protein can be captured from a liquid culture medium using at least one chromatography column and/or chromatographic membrane (e.g., any of the chromatography columns and/or chromatographic membranes described herein).

The term “purifying” means a step performed to isolate a recombinant therapeutic protein from one or more other impurities (e.g., bulk impurities) or components present in a fluid containing a recombinant therapeutic protein (e.g., liquid culture medium proteins or one or more other components (e.g., DNA, RNA, other proteins, endotoxins, viruses, etc.) present in or secreted from a mammalian cell). For example, purifying can be performed during or after an initial capturing step. Purification can be performed using a resin, membrane, or any other solid support that binds either a recombinant therapeutic protein or contaminants (e.g., through the use of affinity chromatography, hydrophobic interaction chromatography, anion or cation exchange chromatography, or molecular sieve chromatography). A recombinant therapeutic protein can be purified from a fluid containing the recombinant therapeutic protein using at least one chromatography column and/or chromatographic membrane (e.g., any of the chromatography columns or chromatographic membranes described herein).

The term “polishing” is a term of art and means a step performed to remove remaining trace or small amounts of contaminants or impurities from a fluid containing a recombinant therapeutic protein that is close to a final desired purity. For example, polishing can be performed by passing a fluid containing the recombinant therapeutic protein through a chromatographic column(s) or membrane

absorber(s) that selectively binds to either the target recombinant therapeutic protein or small amounts of contaminants or impurities present in a fluid containing a recombinant therapeutic protein. In such an example, the eluate/filtrate of the chromatographic column(s) or membrane absorber(s) contains the recombinant therapeutic protein.

The term “eluate/filtrate” is a term of art and means a fluid that is emitted from a chromatography column or chromatographic membrane that contains a detectable amount of a recombinant therapeutic protein.

The term “filtering” means the removal of at least part of (e.g., at least 80%, 90%, 95%, 96%, 97%, 98%, or 99%) undesired biological contaminants (e.g., a mammalian cell, bacteria, yeast cells, viruses, or mycobacteria) and/or particulate matter (e.g., precipitated proteins) from a liquid (e.g., a liquid culture medium or fluid present in any of the systems or processes described herein).

The term “secreted protein” or “secreted recombinant protein” means a protein (e.g., a recombinant protein) that originally contained at least one secretion signal sequence when it is translated within a mammalian cell, and through, at least in part, enzymatic cleavage of the secretion signal sequence in the mammalian cell, is secreted at least partially into the extracellular space (e.g., a liquid culture medium). Skilled practitioners will appreciate that a “secreted” protein need not dissociate entirely from the cell to be considered a secreted protein.

The term “perfusion bioreactor” means a bioreactor containing a plurality of cells (e.g., mammalian cells) in a first liquid culture medium, wherein the culturing of the cells present in the bioreactor includes periodic or continuous removal of the first liquid culture medium and at the same time or shortly thereafter adding substantially the same volume of a second liquid culture medium to the bioreactor. In some examples, there is an incremental change (e.g., increase or decrease) in the volume of the first liquid culture medium removed and added over incremental periods (e.g., an about 24-hour period, a period of between about 1 minute and about 24-hours, or a period of greater than 24 hours) during the culturing period (e.g., the culture medium refeed rate on a daily basis). The fraction of media removed and replaced each day can vary depending on the particular cells being cultured, the initial seeding density, and the cell density at a particular time. “RV” or “reactor volume” means the volume

of the culture medium present at the beginning of the culturing process (e.g., the total volume of the culture medium present after seeding).

The term “fed-batch bioreactor” is a term of art and means a bioreactor containing a plurality of cells (e.g., mammalian cells) in a first liquid culture medium, wherein the culturing of the cells present in the bioreactor includes the periodic or continuous addition of a second liquid culture medium to the first liquid culture medium without substantial or significant removal of the first liquid culture medium or second liquid culture medium from the cell culture. The second liquid culture medium can be the same as the first liquid culture medium. In some examples of fed-batch culture, the second liquid culture medium is a concentrated form of the first liquid culture medium. In some examples of fed-batch culture, the second liquid culture medium is added as a dry powder.

The term “clarified liquid culture medium” means a liquid culture medium obtained from a bacterial or yeast cell culture that is substantially free (e.g., at least 80%, 85%, 90%, 92%, 94%, 96%, 98%, or 99% free) of bacteria or yeast cells.

The term “unit operation” is a term of art and means a functional step that can be performed in a process of manufacturing a therapeutic protein drug substance from a liquid culture medium. For example, a unit of operation can be filtering (e.g., removal of contaminant bacteria, yeast viruses, or mycobacteria, and/or particular matter from a fluid containing a recombinant therapeutic protein), capturing, epitope tag removal, purifying, holding or storing, polishing, viral inactivating, adjusting the ionic concentration and/or pH of a fluid containing the recombinant therapeutic protein, and removing unwanted salts.

“Specific productivity rate” or “SPR” is a term of art and as used herein refers to the mass or enzymatic activity of a recombinant therapeutic protein produced per mammalian cell per day. The SPR for a recombinant therapeutic antibody is usually measured as mass/cell/day. The SPR for a recombinant therapeutic enzyme is usually measured as units/cell/day or (units/mass)/cell/day.

“Volume productivity rate” or “VPR” is a term of art and as used herein refers to the mass or enzymatic activity of recombinant therapeutic protein produced per volume of culture (e.g., per L of bioreactor, vessel, or tube volume) per day. The VPR for a recombinant therapeutic antibody is usually measured as mass/L/day. The

VPR for a recombinant therapeutic enzyme is usually measured as units/L/day or mass/L/day.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 is a schematic showing a two-PCCS manufacturing system connected with a perfusion culture bioreactor that results in the continuous manufacture of a therapeutic protein drug substance. The asterisk indicates an exemplary position where one or both of a circuit system including a tangential flow filtration (TFF) unit (e.g., any of the circuit systems including a TFF unit described herein) and a circuit system including a tangential flow virus filtration (TFVF) unit (e.g., any of the circuit systems including a TFVF unit described herein) can be positioned in this exemplary manufacturing system.

Figure 2 is a schematic showing a manufacturing system connected with a perfusion culture bioreactor that results in the continuous manufacture of a therapeutic protein drug substance. The asterisks indicate exemplary positions where one or both of a circuit system including a TFF unit (e.g., any of the circuit systems including a TFF unit described herein) and a circuit system including a tangential flow virus filtration (TFVF) unit (e.g., any of the circuit systems including a TFVF unit described herein) can be positioned in this exemplary manufacturing system.

Figure 3 is a diagram of a PCC system cycle containing three chromatography columns. At the beginning of a cycle, the feed solution is loaded onto column 1, and the flow through goes to waste until product breakthrough occurs (step 1). At this

point, the flow through from column 1 is directed to column 2 to capture the unbound recombinant therapeutic protein from column 1 (step 2). Once column 1 is fully loaded, the feed is now directed loaded onto column 2, while column 1 is washed, eluted, regenerated, and re-equilibrated for the next cycle (steps 3 and 4). Column 2
 5 now goes through steps 3-5, which are identical to steps 1-3 for column 1. Finally, column 3 goes through steps 5-6, the same as columns 1 and 2. Once all three columns have completed these steps, the cycle restarts with column 1.

Figure 4 is a schematic diagram demonstrating the principle of column switching based on ΔUV . T1 designates the time when the ΔUV has reached a pre-determined threshold level. Once this threshold level is reached, the flow through
 10 from column 1 is directed onto column 2 rather than to the waste. T2 designates the time when the column has been saturated with product. The ΔUV value for both T1 and T2 are process specific.

Figure 5 is a schematic of the circuit system including a tangential flow
 15 filtration unit during a first period of time, when a fluid including a recombinant therapeutic protein is continuously flowed through into the system (“transfer”) and a filtrate not including the recombinant therapeutic protein is discarded (“permeate”) (left panel) and a graph showing the mass flux (g/h) over time (h) during the first period of time (right panel). The recombinant therapeutic protein is retained within
 20 the circuit system and is recirculated through the circuit system (“retentate”) during the first period of time.

Figure 6 is a schematic of the circuit system including a tangential flow
 filtration unit during a second period of time, when a diafiltration medium is continuously flowed through into the system (“DF buffer”) and a filtrate not including
 25 the recombinant therapeutic protein is discarded (“permeate”) (left panel) and a graph showing the mass flux (g/h) over time (h) during the second period of time (right panel). The recombinant therapeutic protein is retained within the circuit system and is recirculated through the circuit system (“retentate”) during the second period of time.

30 Figure 7 is a graph illustrating the results of the use of an exemplary circuit system including a tangential flow filtration unit to perform ultrafiltration/diafiltration of a fluid including a recombinant therapeutic protein. The flux (LMH), the fold-concentration of the recombinant therapeutic protein (Fold), the volume of fluid in the

circuit system, the diavolumes, and transmembrane pressure (TMP) of the circuit system including a tangential flow filtration unit over time are shown.

Figure 8 is a graph illustrating the results of the use of an exemplary circuit system including a tangential flow filtration unit to perform ultrafiltration/diafiltration of a fluid including a recombinant therapeutic protein. The flux (LMH), the fold-
5 concentration of the recombinant therapeutic protein (Fold), the volume of fluid in the circuit system, the diavolumes, and transmembrane pressure (TMP) of the circuit system including a tangential flow filtration unit over time are shown.

Figure 9 is a graph illustrating the results of the use of an exemplary circuit system including a tangential flow filtration unit to perform ultrafiltration/diafiltration of a fluid including a recombinant therapeutic protein. The flux (LMH), the fold-
10 concentration of the recombinant therapeutic protein (Fold), the volume of fluid in the circuit system, the diavolumes, and transmembrane pressure (TMP) of the circuit system including a tangential flow filtration unit over time are shown.

Figure 10 is a schematic of the circuit system including a tangential flow virus filtration unit, when a fluid including a recombinant therapeutic protein is continuously flowed into the system (“from UF”) and a filtrate including the recombinant therapeutic protein is continuously collected (“VF product”).
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Figure 11 is a schematic showing an exemplary circuit system including a tangential flow virus filtration unit.
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Figure 12 is a graph showing the data obtained from the use of a circuit system including a tangential flow virus filtration unit to perform virus filtration. The black line represents the pressure (psi) and the gray line represents the mass of the permeate (kg) over time.

Figure 13 is a graph illustrating the results of the use of an exemplary circuit system including a TFVF unit to perform viral filtration of Murine Minute Virus (MMV) from a recombinant human antibody (mAb) using a Planova™ 20N filter operated at 12 psi with a varying recirculation rate from 1-2 mL/min. The mass and pressure profiles of the circuit system including a TFVF unit over 2.5 hours are
25 shown.
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Figure 14 is a graph illustrating the results of the use of an exemplary circuit system including a TFVF unit to perform continuous viral filtration of Murine Minute Virus (MMV) from a recombinant human antibody (mAb) using a Planova™ 20N

filter operated at 6.6 psi with a varying recirculation rate of 2 mL/min. The mass and pressure profiles of the TFVF unit over 22 hours are shown.

Figure 15 is a graph illustrating the results of the use of an exemplary circuit system including a TFVF unit to perform continuous viral filtration of Murine MinuteVirus (MMV) from a recombinant human antibody (mAb) with refeed using a Planova™ BioEx (3 cm² area) operated at 19.8 psi with a varying recirculation rate of 1 mL/min. The mass and pressure profiles of the TFVF unit over 29 hours are shown.

Figure 16 is a graph illustrating the results of the use of an exemplary circuit system including a TFVF unit to perform continuous viral filtration of Murine Minute Virus (MMV) from a recombinant human antibody (mAb) with refeed using a Planova™ BioEx (10 cm² area) operated at 19.8 psi with a varying recirculation rate of 3 mL/min. The mass and pressure profiles of the TFVF unit over 29 hours are shown.

DETAILED DESCRIPTION

Provided herein are methods of processing a fluid including a recombinant therapeutic protein that include (a) providing a circuit system including (i) a tangential flow filtration (TFF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets, including at least one port for flowing fluid into or out of, or both, of the system, where the system is configured such that fluid can be flowed through the conduit and the TFF unit, and filtrate not including the recombinant therapeutic protein can be collected from the TFF unit; (b) continuously flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port, and discarding filtrate not including the recombinant therapeutic protein for a first period of time; (c) continuously flowing a diafiltration medium into the circuit system through one of the at least one port, and discarding filtrate not including the recombinant therapeutic protein for a second period of time; and (d) collecting a fluid including the recombinant therapeutic protein that is present in the circuit system after the first and second time periods through one of the at least one port. Also provided are methods of processing a fluid including a recombinant therapeutic protein that include: (a) providing a circuit system including (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a conduit in fluid communication between the

first and second inlets of the TFVF unit, including at least one port for flowing fluid into or out of, or both, of the system, where the system is configured such that fluid can be flowed through the conduit and the TFVF unit, and filtrate including the recombinant therapeutic protein can be collected from the TFVF unit; and (b)

5 continuously flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port, and collecting filtrate comprising the recombinant therapeutic protein from the TFVF unit for a period of time. As can be appreciated by those skilled in the art, some embodiments of these methods can further include recirculating any fluid including the recombinant therapeutic protein in

10 the conduit of the circuit system including a TFVF unit that did not pass through the one or more tangential virus filters in the TFVF unit (e.g., acts as a buffer when a new volume of a fluid including the recombinant therapeutic protein is flowed into the conduit of the circuit system including a TFVF unit).

Also provided are integrated and continuous processes for manufacturing a

15 therapeutic protein drug substance that include: (a) providing a liquid culture medium including a recombinant therapeutic protein that is substantially free of cells, wherein the liquid culture medium is fed into a first multi-column chromatography system (MCCS1); (b) capturing the recombinant therapeutic protein in the liquid culture medium using the MCCS1, where the eluate of the MCCS1 containing the

20 recombinant therapeutic protein is continuously fed into a second multi-column chromatography system (MCCS2); (c) purifying and polishing the recombinant therapeutic protein using the MCCS2, where the eluate from the MCCS2 is a fluid including the recombinant therapeutic protein; (d) processing the fluid including the recombinant therapeutic protein using a method of any of the methods described

25 herein, where the process is integrated and runs continuously from said liquid culture medium to the collected fluid or filtrate comprising the recombinant therapeutic protein, wherein the collected fluid or filtrate is the therapeutic protein drug substance.

30 ***Methods of Processing a Fluid Including a Recombinant Therapeutic Protein that Include the Use of a Circuit System Including a TFF Unit***

Provided herein are methods of processing a fluid including a recombinant therapeutic protein that include: providing a circuit system including: (a) a tangential

flow filtration (TFF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets, including at least one port for flowing fluid into or out of, or both, of the system; (b) continuously flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port, and discarding filtrate not including the recombinant therapeutic protein for a first period of time; (c) continuously flowing a diafiltration medium into the circuit system through one of the at least one port, and discarding filtrate not including the recombinant therapeutic protein for a second period of time; and (d) collecting a fluid including the recombinant therapeutic protein that is present in the circuit system after the first and second time points through one of the at least one port. As can be appreciated by those skilled in the art, some embodiments of these methods include recirculating any fluid including the recombinant therapeutic protein in the conduit of the circuit system including a TFF unit that did not pass through the one or more tangential filters in the TFF unit (e.g., acts as a buffer when a new volume of a fluid including the recombinant therapeutic protein and/or a diafiltration medium is flowed into the conduit of the circuit system including a TFF unit).

In some embodiments, the TFF unit includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve tangential filters. In some examples of these methods, the TFF unit can include one or more tangential filter(s) having a surface area of about 0.05 cm² to about 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², about 40 cm², about 38 cm², about 36 cm², about 34 cm², about 32 cm², about 30 cm², about 28 cm², about 26 cm², about 24 cm², about 22 cm², about 20 cm², about 18 cm², about 16 cm², about 14 cm², about 12 cm², about 10 cm², about 9.5 cm², about 9.0 cm², about 8.5 cm², about 8.0 cm², about 7.5 cm², about 7.0 cm², about 6.5 cm², about 6.0 cm², about 5.5 cm², about 5.0 cm², about 4.5 cm², about 4.0 cm², about 3.5 cm², about 3.0 cm², about 2.5 cm², about 2.0 cm², about 1.5 cm², about 1.0 cm², about 0.5 cm², or about 0.1 cm²; about 0.1 cm² to about 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², about 40 cm², about 38 cm², about 36 cm², about 34 cm², about 32 cm², about 30 cm², about 28 cm², about 26 cm², about 24 cm², about 22 cm², about 20 cm², about 18 cm², about 16 cm², about 14 cm², about 12 cm², about 10 cm², about 9.5 cm², about 9.0 cm², about 8.5 cm², about 8.0 cm², about 7.5 cm², about 7.0 cm², about 6.5 cm², about 6.0 cm², about 5.5 cm², about 5.0 cm², about 4.5 cm², about 4.0 cm², about 3.5 cm², about 3.0 cm², about 2.5 cm², about 2.0 cm²,

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about 36 cm², about 34 cm², or about 32 cm²; about 32 cm² to about 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², about 40 cm², about 38 cm², about 36 cm², or about 34 cm²; about 34 cm² to about 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², about 40 cm², about 38 cm², or about 36 cm²; about 36 cm² to about 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², about 40 cm², or about 38 cm²; about 38 cm² to about 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², or about 40 cm²; about 40 cm² to about 50 cm², about 48 cm², about 46 cm², about 44 cm², or about 42 cm²; about 42 cm² to about 50 cm², about 48 cm², about 46 cm², or about 44 cm²; about 44 cm² to about 50 cm², about 48 cm², or about 46 cm²; about 46 cm² to about 50 cm² or about 48 cm²; or about 48 cm² to about 50 cm².

In some embodiments, the one or more tangential filters in the TFF unit can have a molecular weight cut-off of about 1 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about 14,000 kDa, about 12,000 kDa, about 10,000 kDa, about 8,000 kDa, about 6,000 kDa, about 4,000 kDa, about 2,000 kDa, about 1,800 kDa, about 1,600 kDa, about 1,400 kDa, about 1,200 kDa, about 1,000 kDa, about 950 kDa, about 900 kDa, about 850 kDa, about 800 kDa, about 750 kDa, about 700 kDa, about 650 kDa, about 600 kDa, about 550 kDa, about 500 kDa, about 450 kDa, about 400 kDa, about 350 kDa, about 300 kDa, about 250 kDa, about 200 kDa, about 150 kDa, about 100 kDa, about 80 kDa, about 60 kDa, about 40 kDa, about 20 kDa, about 10 kDa, or about 5 kDa; about 5 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about 14,000 kDa, about 12,000 kDa, about 10,000 kDa, about 8,000 kDa, about 6,000 kDa, about 4,000 kDa, about 2,000 kDa, about 1,800 kDa, about 1,600 kDa, about 1,400 kDa, about 1,200 kDa, about 1,000 kDa, about 950 kDa, about 900 kDa, about 850 kDa, about 800 kDa, about 750 kDa, about 700 kDa, about 650 kDa, about 600 kDa, about 550 kDa, about 500 kDa, about 450 kDa, about 400 kDa, about 350 kDa, about 300 kDa, about 250 kDa, about 200 kDa, about 150 kDa, about 100 kDa, about 80 kDa, about 60 kDa, about 40 kDa, about 20 kDa, or about 10 kDa; about 10 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about 14,000 kDa, about 12,000 kDa, about 10,000 kDa, about 8,000 kDa, about 6,000 kDa, about 4,000 kDa, about 2,000 kDa, about 1,800 kDa, about

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 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about 14,000 kDa, about 12,000
 15 kDa, about 10,000 kDa, about 8,000 kDa, about 6,000 kDa, about 4,000 kDa, about
 2,000 kDa, about 1,800 kDa, about 1,600 kDa, about 1,400 kDa, about 1,200 kDa,
 about 1,000 kDa, about 950 kDa, about 900 kDa, about 850 kDa, about 800 kDa,
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 500 kDa, or about 450 kDa; about 450 kDa to about 25,000 kDa, about 24,000 kDa,
 20 about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about
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 25 about 550 kDa, or about 500 kDa; about 500 kDa to about 25,000 kDa, about 24,000
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 30 about 850 kDa, about 800 kDa, about 750 kDa, about 700 kDa, about 650 kDa, about
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 about 750 kDa, about 700 kDa, or about 650 kDa; about 650 kDa to about 25,000
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 8,000 kDa, about 6,000 kDa, about 4,000 kDa, about 2,000 kDa, about 1,800 kDa,
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 5 about 1,400 kDa, about 1,200 kDa, about 1,000 kDa, or about 950 kDa; about 950
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 30 about 10,000 kDa, about 8,000 kDa, about 6,000 kDa, or about 4,000 kDa; about
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25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about 14,000 kDa, about 12,000 kDa, about 10,000 kDa, or about 8,000 kDa; about 8,000 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about 14,000 kDa, about 12,000 kDa, or about 10,000 kDa; about 10,000 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, about 14,000 kDa, or about 12,000 kDa; about 12,000 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, about 16,000 kDa, or about 14,000 kDa; about 14,000 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, about 18,000 kDa, or about 16,000 kDa; about 16,000 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, about 20,000 kDa, or about 18,000 kDa; about 18,000 kDa to about 25,000 kDa, about 24,000 kDa, about 22,000 kDa, or about 20,000 kDa; about 20,000 kDa to about 25,000 kDa, about 24,000 kDa, or about 22,000 kDa; about 22,000 kDa to about 25,000 kDa or about 24,000 kDa; or about 23,000 kDa to about 25,000 kDa. In some embodiments, the TFF unit includes one or more tangential filters made of, e.g., cellulose (e.g., regenerated cellulose) or polyethersulfone. Non-limiting examples of one or more tangential filters that can be used in the TFF unit are commercially available from Millipore (e.g., Ultracel[®] and Biomax[®]). Additional commercial sources of one or more tangential filters that can be used in the TFF unit are known in the art.

The conduit can be simple tubing, e.g., biocompatible tubing. Non-limiting examples of useful tubing include silicone rubber, polyurethane, polydioxanone (PDO), polyhydroxyalkanoate, polyhydroxybutyrate, poly(glycerol sebacate), polyglycolide, polylactide, polycaprolactone, or polyanhydride, or copolymers or derivatives including these and/or other polymers. Alternatively or in addition, any of the conduits described herein can include polyvinyl chloride. A conduit can be, e.g., weldable transfer tubing.

In some embodiments, the internal diameter of the conduit can be about 5 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, about 32 mm, about 30 mm, about 28 mm, about 26 mm, about 24 mm, about 22 mm, about 20 mm, about 18 mm, about 16 mm, about 14 mm, about 12 mm, about 10 mm, about 8 mm, or about 6 mm; about 6

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mm, about 34 mm, about 32 mm, about 30 mm, or about 28 mm; about 28 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, about 32 mm, or about 30 mm; about 30 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, or about 32 mm; about 32 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, or about 34 mm; about 34 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, or about 36 mm; about 36 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, to about 38 mm; about 38 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, or about 40 mm; about 40 mm to about 50 mm, about 48 mm, about 46 mm, about 44 mm, or about 42 mm; about 42 mm to about 50 mm, about 48 mm, about 46 mm, or about 44 mm; about 44 mm to about 50 mm, about 48 mm, or about 46 mm; about 46 mm to about 50 mm or about 48 mm; or about 48 mm to about 50 mm.

In some embodiments, the conduit has a total length of about 1.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, about 10 cm, about 8.0 cm, about 6.0 cm, about 4.0 cm, or about 2.0 cm; about 2.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, about 10 cm, about 8.0 cm, about 6.0 cm, or about 4.0 cm; about 4.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, about 10 cm, about 8.0 cm, or about 6.0 cm; about 6.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, about 10 cm, or about 8.0 cm; about 8.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, or about 10 cm; about 10 cm to about 75 cm, about 70 cm, about 65 cm,

about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm,
 about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm, about 14 cm, or
 about 12 cm; about 12 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm,
 about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm,
 5 about 25 cm, about 20 cm, about 18 cm, about 16 cm, or about 14 cm; about 14 cm to
 about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm,
 about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm,
 about 18 cm, or about 16 cm; about 16 cm to about 75 cm, about 70 cm, about 65 cm,
 about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm,
 10 about 30 cm, about 25 cm, about 20 cm, or about 18 cm; about 18 cm to about 75 cm,
 about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm,
 about 40 cm, about 35 cm, about 30 cm, about 25 cm, or about 20 cm; about 20 cm to
 about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm,
 about 45 cm, about 40 cm, about 35 cm, about 30 cm, or about 25 cm; about 25 cm to
 15 about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm,
 about 45 cm, about 40 cm, about 35 cm, or about 30 cm; about 30 cm to about 75 cm,
 about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm,
 about 40 cm, or about 35 cm; about 35 cm to about 75 cm, about 70 cm, about 65 cm,
 about 60 cm, about 55 cm, about 50 cm, about 45 cm, or about 40 cm; about 40 cm to
 20 about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, or
 about 45 cm; about 45 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm,
 about 55 cm, or about 50 cm; about 50 cm to about 75 cm, about 70 cm, about 65 cm,
 about 60 cm, or about 55 cm; about 55 cm to about 75 cm, about 70 cm, about 65 cm,
 or about 60 cm; about 60 cm to about 75 cm, about 70 cm, or about 65 cm; about 65
 25 cm to about 75 cm or about 70 cm; or about 70 cm to about 75 cm.

Additional examples of conduits and properties of conduits that can be used in the circuit system are well-known by those in the art.

In some examples, the circuit system includes one, two, three, four, or five
 ports. In some examples, the at least one port can be a valve. In other examples, the
 30 at least one port can be an injection port or can have a ribbed threading. The at least
 one port can be any type of port commonly known in the art.

In some examples, the circuit system can further include a pump (e.g., a low-turbulence pump, e.g., a peristaltic pump) disposed in the conduit.

In some examples, step (b) includes continuously flowing (e.g., unidirectionally or bidirectionally flowing) the fluid including the recombinant therapeutic protein into the circuit system at a rate of between about 0.05 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute, about 20 mL/minute, about 18 mL/minute, about 16 mL/minute, about 14 mL/minute, about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0 mL/minute, about 8.5 mL/minute, about 8.0 mL/minute, about 7.5 mL/minute, about 7.0 mL/minute, about 6.5 mL/minute, about 6.0 mL/minute, about 5.5 mL/minute, about 5.0 mL/minute, about 4.5 mL/minute, about 4.0 mL/minute, about 3.5 mL/minute, about 3.0 mL/minute, about 2.5 mL/minute, about 2.0 mL/minute, about 1.5 mL/minute, about 1.0 mL/minute, about 0.5 mL/minute, or about 0.1 mL/minute; about 0.1 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute, about 20 mL/minute, about 18 mL/minute, about 16 mL/minute, about 14 mL/minute, about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0 mL/minute, about 8.5 mL/minute, about 8.0 mL/minute, about 7.5 mL/minute, about 7.0 mL/minute, about 6.5 mL/minute, about 6.0 mL/minute, about 5.5 mL/minute, about 5.0 mL/minute, about 4.5 mL/minute, about 4.0 mL/minute, about

3.5 mL/minute, about 3.0 mL/minute, about 2.5 mL/minute, about 2.0 mL/minute,
 about 1.5 mL/minute, about 1.0 mL/minute, or about 0.5 mL/minute; about 0.5
 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70
 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30
 5 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800
 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute,
 about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450
 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute,
 10 about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100
 mL/minute, about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22
 mL/minute, about 20 mL/minute, about 18 mL/minute, about 16 mL/minute, about 14
 mL/minute, about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about
 9.0 mL/minute, about 8.5 mL/minute, about 8.0 mL/minute, about 7.5 mL/minute,
 15 about 7.0 mL/minute, about 6.5 mL/minute, about 6.0 mL/minute, about 5.5
 mL/minute, about 5.0 mL/minute, about 4.5 mL/minute, about 4.0 mL/minute, about
 3.5 mL/minute, about 3.0 mL/minute, about 2.5 mL/minute, about 2.0 mL/minute,
 about 1.5 mL/minute, or about 1.0 mL/minute; about 1.0 mL/minute to about 100
 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60
 20 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
 25 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute,
 about 20 mL/minute, about 18 mL/minute, about 16 mL/minute, about 14 mL/minute,
 about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0
 30 mL/minute, about 8.5 mL/minute, about 8.0 mL/minute, about 7.5 mL/minute, about
 7.0 mL/minute, about 6.5 mL/minute, about 6.0 mL/minute, about 5.5 mL/minute,
 about 5.0 mL/minute, about 4.5 mL/minute, about 4.0 mL/minute, about 3.5
 mL/minute, about 3.0 mL/minute, about 2.5 mL/minute, about 2.0 mL/minute, or

about 1.5 mL/minute; about 1.5 mL/minute to about 100 L/minute, about 90
 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50
 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 5 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute,
 about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
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 18 mL/minute, about 16 mL/minute, about 14 mL/minute, about 12 mL/minute, about
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 15 4.5 mL/minute, about 4.0 mL/minute, about 3.5 mL/minute, about 3.0 mL/minute,
 about 2.5 mL/minute, or about 2.0 mL/minute; about 2.0 mL/minute to about 100
 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60
 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 20 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 25 about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute,
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 about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0
 mL/minute, about 8.5 mL/minute, about 8.0 mL/minute, about 7.5 mL/minute, about
 7.0 mL/minute, about 6.5 mL/minute, about 6.0 mL/minute, about 5.5 mL/minute,
 30 about 5.0 mL/minute, about 4.5 mL/minute, about 4.0 mL/minute, about 3.5
 mL/minute, about 3.0 mL/minute, or about 2.5 mL/minute; about 2.5 mL/minute to
 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about
 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20

L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
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 5 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute,
 about 20 mL/minute, about 18 mL/minute, about 16 mL/minute, about 14 mL/minute,
 about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0
 10 mL/minute, about 8.5 mL/minute, about 8.0 mL/minute, about 7.5 mL/minute, about
 7.0 mL/minute, about 6.5 mL/minute, about 6.0 mL/minute, about 5.5 mL/minute,
 about 5.0 mL/minute, about 4.5 mL/minute, about 4.0 mL/minute, about 3.5
 mL/minute, or about 3.0 mL/minute; about 3.0 mL/minute to about 100 L/minute,
 about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about
 15 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute,
 about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550
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 20 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
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 25 mL/minute, about 24 mL/minute, about 22 mL/minute, about 20 mL/minute, about
 18 mL/minute, about 16 mL/minute, about 14 mL/minute, about 12 mL/minute, about
 10 mL/minute, about 9.5 mL/minute, about 9.0 mL/minute, about 8.5 mL/minute,
 25 about 8.0 mL/minute, about 7.5 mL/minute, about 7.0 mL/minute, about 6.5
 mL/minute, about 6.0 mL/minute, about 5.5 mL/minute, about 5.0 mL/minute, about
 4.5 mL/minute, about 4.0 mL/minute, or about 3.5 mL/minute; about 3.5 mL/minute
 to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute,
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 30 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
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about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
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 5 about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0
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 mL/minute, about 850 mL/minute, or about 800 mL/minute; about 800 mL/minute to
 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about

60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, or about 850 mL/minute; about 850 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, or about 950 mL/minute; about 900 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, or about 950 mL/minute; about 950 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, or about 1 L/minute; about 1 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, or about 10 L/minute; about 10 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, or about 20 L/minute; about 20 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, or about 30 L/minute; about 30 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, or about 40 L/minute; about 40 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, or about 50 L/minute; about 50 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, or about 60 L/minute; about 60 L/minute to about 100 L/minute, about 90 L/minute, or about 80 L/minute; about 70 L/minute to about 100 L/minute, about 90 L/minute, or about 80 L/minute; about 80 L/minute to about 100 L/minute, or about 90 L/minute; or about 90 L/minute to about 100 L/minute.

In some examples, step (b) includes continuously flowing (e.g., unidirectionally or bidirectionally flowing) a fluid including about 0.01 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, about 5.0 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, about 3.5 mg/mL, about 3.0 mg/mL, about 2.5 mg/mL, about 2.0 mg/mL, about 1.5 mg/mL, about 1.0 mg/mL, about 0.5 mg/mL, about 0.1 mg/mL, or about 0.05 mg/mL; about 0.05 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, about 5.0 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, about 3.5 mg/mL, about 3.0 mg/mL, about 2.5 mg/mL, about 2.0 mg/mL, about 1.5 mg/mL, about 1.0 mg/mL, about 0.5 mg/mL, or about 0.1 mg/mL; about 0.1 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, about 5.0 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, about 3.5 mg/mL, about 3.0 mg/mL, about 2.5 mg/mL, about 2.0 mg/mL, about 1.5 mg/mL, about 1.0 mg/mL, or about 0.5 mg/mL; about 0.5 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18

mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5
 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL,
 about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, about 5.0
 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, about 3.5 mg/mL, about 3.0 mg/mL,
 5 about 2.5 mg/mL, about 2.0 mg/mL, about 1.5 mg/mL, or about 1.0 mg/mL; about 1.0
 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL,
 10 about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL,
 about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5
 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, about 5.0 mg/mL, about 4.5 mg/mL,
 about 4.0 mg/mL, about 3.5 mg/mL, about 3.0 mg/mL, about 2.5 mg/mL, about 2.0
 mg/mL, or about 1.5 mg/mL; about 1.5 mg/mL to about 50 mg/mL, about 48 mg/mL,
 15 about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38
 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about
 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL,
 about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10
 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5
 20 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL,
 about 5.0 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, about 3.5 mg/mL, about 3.0
 mg/mL, about 2.5 mg/mL, or about 2.0 mg/mL; about 2.0 mg/mL to about 50 mg/mL,
 about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40
 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about
 25 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL,
 about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12
 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0
 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL,
 about 5.5 mg/mL, about 5.0 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, about 3.5
 30 mg/mL, about 3.0 mg/mL, or about 2.5 mg/mL; about 2.5 mg/mL to about 50 mg/mL,
 about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40
 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about
 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL,

about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12
 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0
 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL,
 about 5.5 mg/mL, about 5.0 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, about 3.5
 5 mg/mL, or about 3.0 mg/mL; about 3.0 mg/mL to about 50 mg/mL, about 48 mg/mL,
 about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38
 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about
 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL,
 about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10
 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5
 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL,
 about 5.0 mg/mL, about 4.5 mg/mL, about 4.0 mg/mL, or about 3.5 mg/mL; about 3.5
 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 15 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL,
 about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL,
 about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5
 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, about 5.0 mg/mL, about 4.5 mg/mL, or
 20 about 4.0 mg/mL; about 4.0 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46
 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about
 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL,
 about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18
 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5
 25 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL,
 about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, about 5.0
 mg/mL, or about 4.5 mg/mL; about 4.5 mg/mL to about 50 mg/mL, about 48 mg/mL,
 about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38
 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about
 30 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL,
 about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10
 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5
 mg/mL, about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, about 5.5 mg/mL, or

about 5.0 mg/mL; about 5.0 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46
 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about
 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL,
 about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18
 5 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5
 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL,
 about 7.0 mg/mL, about 6.5 mg/mL, about 6.0 mg/mL, or about 5.5 mg/mL; about 5.5
 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 10 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL,
 about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL,
 about 8.5 mg/mL, about 8.0 mg/mL, about 7.5 mg/mL, about 7.0 mg/mL, about 6.5
 mg/mL, or about 6.0 mg/mL; about 6.0 mg/mL to about 50 mg/mL, about 48 mg/mL,
 15 about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38
 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about
 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL,
 about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10
 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about 8.0 mg/mL, about 7.5
 20 mg/mL, about 7.0 mg/mL, or about 6.5 mg/mL; about 6.5 mg/mL to about 50
 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about
 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL,
 about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22
 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about
 25 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about
 8.0 mg/mL, about 7.5 mg/mL, or about 7.0 mg/mL; about 7.0 mg/mL to about 50
 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about
 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL,
 about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22
 30 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about
 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, about
 8.0 mg/mL, or about 7.5 mg/mL; about 7.5 mg/mL to about 50 mg/mL, about 48
 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about

38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0 mg/mL, about 8.5 mg/mL, or about 8.0 mg/mL;

5 about 8.0 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, 9.5 mg/mL, about 9.0

10 mg/mL, or about 8.5 mg/mL; about 8.5 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10

15 mg/mL, 9.5 mg/mL, or about 9.0 mg/mL; about 9.0 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10

20 mg/mL, or 9.5 mg/mL; about 9.5 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, or

25 about 10 mg/mL; about 10 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, or about 12 mg/mL; about 12 mg/mL to

30 about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, or about 14

mg/mL; about 14 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL,
 about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36
 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about
 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, or
 5 about 16 mg/mL; about 16 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46
 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about
 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL,
 about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, or about 18
 mg/mL; about 18 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL,
 10 about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36
 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about
 26 mg/mL, about 24 mg/mL, about 22 mg/mL, or about 20 mg/mL; about 20 mg/mL
 to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42
 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about
 15 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, or
 about 22 mg/mL; about 22 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46
 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about
 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL,
 about 26 mg/mL, or about 24 mg/mL; about 24 mg/mL to about 50 mg/mL, about 48
 20 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about
 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL,
 about 28 mg/mL, or about 26 mg/mL; about 26 mg/mL to about 50 mg/mL, about 48
 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about
 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, or
 25 about 28 mg/mL; about 28 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46
 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about
 36 mg/mL, about 34 mg/mL, about 32 mg/mL, or about 30 mg/mL; about 30 mg/mL
 to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42
 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, or
 30 about 32 mg/mL; about 32 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46
 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about
 36 mg/mL, or about 34 mg/mL; about 34 mg/mL to about 50 mg/mL, about 48
 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about

38 mg/mL, or about 36 mg/mL; about 36 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, or about 38 mg/mL; about 38 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, or about 40 mg/mL; about 40 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, or about 42 mg/mL; about 42 mg/mL to about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, or about 44 mg/mL; about 44 mg/mL to about 50 mg/mL, about 48 mg/mL, or about 46 mg/mL; about 46 mg/mL to about 50 mg/mL or about 48 mg/mL; or about 48 mg/mL to about 50 mg/mL, recombinant therapeutic protein into the circuit system

10 In some embodiments, the first period of time can be about 0.1 minute to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, about 15 minutes, about 10 minutes, about 5 minutes, about 2 minutes, or about 1 minute; about 1 minute to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, about 15 minutes, about 10 minutes, about 5 minutes, or about 2 minutes; about 2 minutes to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, about 15 minutes, about 10 minutes, or about 5 minutes; about 5 minutes to about 12 hours, about 11.5 hours, about 11 hours, about

10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about
 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5
 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0
 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55
 5 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
 about 30 minutes, about 25 minutes, about 20 minutes, about 15 minutes, or about 10
 minutes; about 10 minutes to about 12 hours, about 11.5 hours, about 11 hours, about
 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about
 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5
 10 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0
 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55
 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
 about 30 minutes, about 25 minutes, about 20 minutes, or about 15 minutes; about 15
 minutes to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about
 15 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5
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In some examples, step (c) includes continuously flowing (e.g., unidirectionally or bidirectionally flowing) a diafiltration medium into the circuit system at a rate of between about 0.05 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute, about 20 mL/minute, about

18 mL/minute, about 16 mL/minute, about 14 mL/minute, about 12 mL/minute, about
 10 mL/minute, about 9.5 mL/minute, about 9.0 mL/minute, about 8.5 mL/minute,
 about 8.0 mL/minute, about 7.5 mL/minute, about 7.0 mL/minute, about 6.5
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 5 4.5 mL/minute, about 4.0 mL/minute, about 3.5 mL/minute, about 3.0 mL/minute,
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 mL/minute, about 0.5 mL/minute, or about 0.1 mL/minute; about 0.1 mL/minute to
 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about
 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 10 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 15 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute,
 about 20 mL/minute, about 18 mL/minute, about 16 mL/minute, about 14 mL/minute,
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 25 about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about
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about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0
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 5 mL/minute, about 3.0 mL/minute, about 2.5 mL/minute, about 2.0 mL/minute, about
 1.5 mL/minute, or about 1.0 mL/minute; about 1.0 mL/minute to about 100 L/minute,
 about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about
 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 10 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute,
 about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550
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 25 about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800
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 5 about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about
 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about
 800 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute,
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 10 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute,
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 25 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
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 15 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
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 25 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
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 30 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
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 25 mL/minute, about 24 mL/minute, about 22 mL/minute, about 20 mL/minute, about
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 L/minute to about 100 L/minute.

A diafiltration medium can be, e.g., a physiologically acceptable buffer (e.g.,
 30 phosphate buffered saline or a buffer used to formulate the recombinant therapeutic
 protein, e.g., a buffer comprising one or more of glycine, phosphate, acetate, citrate, a
 bulking agent (e.g., a non-reducing carbohydrate, e.g., sucrose), a surfactant (e.g.,
 polysorbate 80, Triton X-100, and Tween).

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hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0
 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, about 1.5
 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about
 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes,
 5 or about 15 minutes; about 15 minutes to about 12 hours, about 11.5 hours, about 11
 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5
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 5 about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours,
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hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, or about 7.5 hours; about 7.5 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, or about 8.0 hours; about 8.0 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, or about 8.5 hours; about 8.5 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, or about 9.0 hours; about 9.0 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, or about 9.5 hours; about 9.5 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, or about 10 hours; about 10 hours to about 12 hours, about 11.5 hours, about 11 hours, or about 10.5 hours; about 10.5 hours to about 12 hours, about 11.5 hours, or about 11 hours; about 11 hours to about 12 hours, or about 11.5 hours.

In some embodiments, the fluid including the recombinant therapeutic protein that is collected in step (d) includes a concentration of recombinant therapeutic protein of about 0.1 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL, about 7 mg/mL, about 6 mg/mL, about 5 mg/mL, about 4 mg/mL, about 3 mg/mL, about 2.5 mg/mL, about 2.0 mg/mL, about 1.5 mg/mL, about 1.0 mg/mL, or about 0.5 mg/mL; about 0.5 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,

about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL,
 about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL,
 5 about 7 mg/mL, about 6 mg/mL, about 5 mg/mL, about 4 mg/mL, about 3 mg/mL,
 about 2.5 mg/mL, about 2.0 mg/mL, about 1.5 mg/mL, or about 1.0 mg/mL; about 1.0
 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL,
 10 about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64
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 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL,
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 mg/mL, about 8 mg/mL, about 7 mg/mL, about 6 mg/mL, about 5 mg/mL, about 4
 mg/mL, about 3 mg/mL, about 2.5 mg/mL, about 2.0 mg/mL, or about 1.5 mg/mL;
 about 1.5 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94
 20 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about
 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL,
 about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66
 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about
 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL,
 25 about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38
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 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL,

about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66
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 10 about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL,
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 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL,
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 20 about 8 mg/mL, about 7 mg/mL, about 6 mg/mL, about 5 mg/mL, about 4 mg/mL, or
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 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL,
 25 about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58
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 10 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
 about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about
 15 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, or about 14 mg/mL;
 about 14 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL,
 about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84
 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about
 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL,
 20 about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56
 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about
 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL,
 about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28
 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about
 25 18 mg/mL, or about 16 mg/mL; about 16 mg/mL to about 100 mg/mL, about 98
 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 30 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
 about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about

22 mg/mL, about 20 mg/mL, or about 18 mg/mL; about 18 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 5 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 10 24 mg/mL, about 22 mg/mL, or about 20 mg/mL; about 20 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 15 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, or about 22 mg/mL; about 22 mg/mL to about 100 mg/mL, about 98
 20 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 25 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
 about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, or about 24 mg/mL;
 about 24 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL,
 about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84
 30 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about
 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL,
 about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56
 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about

46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL,
about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28
mg/mL, or about 26 mg/mL; about 26 mg/mL to about 100 mg/mL, about 98 mg/mL,
96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL,
5 about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78
mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about
68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL,
about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50
mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about
10 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL,
about 30 mg/mL, or about 28 mg/mL; about 28 mg/mL to about 100 mg/mL, about 98
mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
15 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
mg/mL, or about 30 mg/mL; about 30 mg/mL to about 100 mg/mL, about 98 mg/mL,
20 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL,
about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78
mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about
68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL,
about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50
25 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about
40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, or about 32 mg/mL;
about 32 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL,
about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84
mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about
30 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL,
about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56
mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about
46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL,

about 36 mg/mL, or about 34 mg/mL; about 34 mg/mL to about 100 mg/mL, about 98
 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 5 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
 about 40 mg/mL, about 38 mg/mL, or about 36 mg/mL; about 36 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 10 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 15 about 42 mg/mL, about 40 mg/mL, or about 38 mg/mL; about 38 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 20 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, or about 40 mg/mL; about 40 mg/mL to about 100 mg/mL, about 98
 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 25 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, or about 42 mg/mL;
 about 42 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL,
 30 about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84
 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about
 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL,
 about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56

mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about
 46 mg/mL, or about 44 mg/mL; about 44 mg/mL to about 100 mg/mL, about 98
 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 5 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, or about 46 mg/mL; about 46 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 10 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, or about 48 mg/mL; about 48 mg/mL to about 100
 15 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 20 52 mg/mL, or about 50 mg/mL; about 50 mg/mL to about 100 mg/mL, about 98
 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 25 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, or about 52 mg/mL;
 about 52 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL,
 about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84
 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about
 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL,
 30 about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56
 mg/mL, or about 54 mg/mL; about 54 mg/mL to about 100 mg/mL, about 98 mg/mL,
 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL,
 about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78

mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, or about 56 mg/mL; about 56 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, or about 58 mg/mL; about 58 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, or about 60 mg/mL; about 60 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, or about 64 mg/mL; about 64 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, or about 66 mg/mL; about 66 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, or about 68 mg/mL; about 68 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, or about 70 mg/mL; about 70 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,

about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86
 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about
 76 mg/mL, about 74 mg/mL, or about 72 mg/mL; about 72 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 5 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, or about 74 mg/mL; about 74 mg/mL
 to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
 82 mg/mL, about 80 mg/mL, about 78 mg/mL, or about 76 mg/mL; about 76 mg/mL
 10 to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
 82 mg/mL, about 80 mg/mL, or about 78 mg/mL; about 78 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, or
 15 about 80 mg/mL; about 80 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,
 about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86
 mg/mL, about 84 mg/mL, or about 82 mg/mL; about 82 mg/mL to about 100 mg/mL,
 about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL,
 about 88 mg/mL, about 86 mg/mL, or about 84 mg/mL; about 84 mg/mL to about 100
 20 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, or about 86 mg/mL; about 86 mg/mL to about 100 mg/mL,
 about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, or
 about 88 mg/mL; about 88 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,
 about 94 mg/mL, about 92 mg/mL, or about 90 mg/mL; about 90 mg/mL to about 100
 25 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, or about 92 mg/mL; about 92
 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, or about 94 mg/mL; about
 94 mg/mL to about 100 mg/mL, about 98 mg/mL, or 96 mg/mL; about 96 mg/mL to
 about 100 mg/mL or about 98 mg/mL; or about 98 mg/mL to about 100 mg/mL.

In some embodiments, these fold-diafiltration achieved after the first and
 30 second time periods is about 1-fold to about 20-fold, about 19-fold, about 18-fold,
 about 17-fold, about 16-fold, about 15-fold, about 14-fold, about 13-fold, about 12-
 fold, about 11-fold, about 10-fold, about 9-fold, about 8-fold, about 7-fold, about 6-
 fold, about 5-fold, about 4-fold, about 3-fold, or about 2-fold; about 2-fold to about

20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, about 13-fold, about 12-fold, about 11-fold, about 10-fold, about 9-fold, about 8-fold, about 7-fold, about 6-fold, about 5-fold, about 4-fold, or about 3-fold; about 3-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, about 13-fold, about 12-fold, about 11-fold, about 10-fold, about 9-fold, about 8-fold, about 7-fold, about 6-fold, about 5-fold, or about 4-fold; about 4-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, about 13-fold, about 12-fold, about 11-fold, about 10-fold, about 9-fold, about 8-fold, about 7-fold, about 6-fold, or about 5-fold; about 5-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, about 13-fold, about 12-fold, about 11-fold, about 10-fold, about 9-fold, about 8-fold, or about 7-fold; about 7-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, about 13-fold, about 12-fold, about 11-fold, about 10-fold, or about 9-fold; about 9-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, about 13-fold, about 12-fold, or about 11-fold; about 11-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, or about 13-fold; about 12-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, about 14-fold, or about 13-fold; about 13-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, about 15-fold, or about 14-fold; about 14-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, about 16-fold, or about 15-fold; about 15-fold to about 20-fold, about 19-fold, about 18-fold, about 17-fold, or about 16-fold; about 16-fold to about 20-fold, about 19-fold, about 18-fold, or about 17-fold; about 17-fold to

about 20-fold, about 19-fold, or about 18-fold; about 18-fold to about 20-fold or about 19-fold; or about 19-fold to about 20-fold.

In some embodiments, the circuit system does not include a holding tank.

In other embodiments of this method, during the first period of time, a fluid including the recombinant therapeutic protein and the diafiltration medium are continuously flowed together into the circuit system, and then during the second period of time, only the diafiltration medium is continuously flowed into the circuit system.

The fluid can be collected in step (d) using a syringe, gravity, or a pump (e.g., a peristaltic pump disposed in the conduit). Additional means for collecting the fluid including the recombinant therapeutic protein in step (d) are known in the art.

Some examples of these methods further include: (e) providing a second circuit system including (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets of the TFVF unit, including at least one port for flowing fluid into or out of, or both, of the second system, where the second system is configured such that fluid can be flowed through the conduit and the TFVF unit, and filtrate including the recombinant therapeutic protein can be collected from the TFVF unit; and (f) flowing the collected fluid of step (d) into the second system using a connecting conduit in fluid communication between a port in the system and a port in the second system, and collecting filtrate including the recombinant therapeutic protein from the TFVF unit for a third period of time. As can be appreciated by those skilled in the art, some embodiments of these methods can further include recirculating any fluid including the recombinant therapeutic protein in the conduit of the second circuit system (including a TFVF unit) that did not pass through the one or more tangential virus filters in the TFVF unit (e.g., acts as a buffer when a new volume of a fluid including the recombinant therapeutic protein is flowed into the conduit of the circuit system including a TFVF unit).

In some embodiments, the TFVF unit includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve tangential virus filters. In some examples of these methods, the TFVF unit can include one or more tangential virus filter(s) having a surface area of about 0.05 cm² to about 100 m², about 90 m², about 80 m², about 70 m², about 60 m², about 50 m², about 45 m², about 40 m², about 35 m², about 30 m²,

about 25 m², about 20 m², about 15 m², about 10 m², about 5 m², about 1 m², about
 800 cm², about 600 cm², about 400 cm², about 200 cm², about 150 cm², about 100
 cm², about 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², about 40
 cm², about 38 cm², about 36 cm², about 34 cm², about 32 cm², about 30 cm², about 28
 5 cm², about 26 cm², about 24 cm², about 22 cm², about 20 cm², about 18 cm², about 16
 cm², about 14 cm², about 12 cm², about 10 cm², about 9.5 cm², about 9.0 cm², about
 8.5 cm², about 8.0 cm², about 7.5 cm², about 7.0 cm², about 6.5 cm², about 6.0 cm²,
 about 5.5 cm², about 5.0 cm², about 4.5 cm², about 4.0 cm², about 3.5 cm², about 3.0
 cm², about 2.5 cm², about 2.0 cm², about 1.5 cm², about 1.0 cm², about 0.5 cm², or
 10 about 0.1 cm²; about 0.1 cm² to about 100 m², about 90 m², about 80 m², about 70 m²,
 about 60 m², about 50 m², about 45 m², about 40 m², about 35 m², about 30 m², about
 25 m², about 20 m², about 15 m², about 10 m², about 5 m², about 1 m², about 800 cm²,
 about 600 cm², about 400 cm², about 200 cm², about 150 cm², about 100 cm², about
 50 cm², about 48 cm², about 46 cm², about 44 cm², about 42 cm², about 40 cm², about
 15 38 cm², about 36 cm², about 34 cm², about 32 cm², about 30 cm², about 28 cm², about
 26 cm², about 24 cm², about 22 cm², about 20 cm², about 18 cm², about 16 cm², about
 14 cm², about 12 cm², about 10 cm², about 9.5 cm², about 9.0 cm², about 8.5 cm²,
 about 8.0 cm², about 7.5 cm², about 7.0 cm², about 6.5 cm², about 6.0 cm², about 5.5
 cm², about 5.0 cm², about 4.5 cm², about 4.0 cm², about 3.5 cm², about 3.0 cm², about
 20 2.5 cm², about 2.0 cm², about 1.5 cm², about 1.0 cm², or about 0.5 cm²; about 0.5 cm²
 to about 100 m², about 90 m², about 80 m², about 70 m², about 60 m², about 50 m²,
 about 45 m², about 40 m², about 35 m², about 30 m², about 25 m², about 20 m², about
 15 m², about 10 m², about 5 m², about 1 m², about 800 cm², about 600 cm², about 400
 cm², about 200 cm², about 150 cm², about 100 cm², about 50 cm², about 48 cm²,
 25 about 46 cm², about 44 cm², about 42 cm², about 40 cm², about 38 cm², about 36 cm²,
 about 34 cm², about 32 cm², about 30 cm², about 28 cm², about 26 cm², about 24 cm²,
 about 22 cm², about 20 cm², about 18 cm², about 16 cm², about 14 cm², about 12 cm²,
 about 10 cm², about 9.5 cm², about 9.0 cm², about 8.5 cm², about 8.0 cm², about 7.5
 cm², about 7.0 cm², about 6.5 cm², about 6.0 cm², about 5.5 cm², about 5.0 cm², about
 30 4.5 cm², about 4.0 cm², about 3.5 cm², about 3.0 cm², about 2.5 cm², about 2.0 cm²,
 about 1.5 cm², or about 1.0 cm²; about 1.0 cm² to about 100 m², about 90 m², about 80
 m², about 70 m², about 60 m², about 50 m², about 45 m², about 40 m², about 35 m²,
 about 30 m², about 25 m², about 20 m², about 15 m², about 10 m², about 5 m², about 1

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about 10 m²; about 10 m² to about 100 m², about 90 m², about 80 m², about 70 m²,
 about 60 m², about 50 m², about 45 m², about 40 m², about 35 m², about 30 m², about
 25 m², about 20 m², or about 15 m²; about 15 m² to about 100 m², about 90 m², about
 80 m², about 70 m², about 60 m², about 50 m², about 45 m², about 40 m², about 35 m²,
 5 about 30 m², about 25 m², or about 20 m²; about 20 m² to about 100 m², about 90 m²,
 about 80 m², about 70 m², about 60 m², about 50 m², about 45 m², about 40 m², about
 35 m², about 30 m², or about 25 m²; about 25 m² to about 100 m², about 90 m², about
 80 m², about 70 m², about 60 m², about 50 m², about 45 m², about 40 m², about 35 m²,
 or about 30 m²; about 30 m² to about 50 m², about 45 m², about 40 m², or about 35
 10 m²; about 35 m² to about 100 m², about 90 m², about 80 m², about 70 m², about 60 m²,
 about 50 m², about 45 m², or about 40 m²; about 40 m² to about 100 m², about 90 m²,
 about 80 m², about 70 m², about 60 m², about 50 m² or about 45 m²; about 45 m² to
 about 100 m², about 90 m², about 80 m², about 70 m², about 60 m², or about 50 m²;
 about 50 m² to about 100 m², about 90 m², about 80 m², about 70 m², or about 60 m²;
 15 about 60 m² to about 100 m², about 90 m², about 80 m², or about 70 m²; about 70 m²
 to about 100 m², about 90 m², or about 80 m²; about 80 m² to about 100 m², or about
 90 m²; or about 90 m² to about 100 m².

In some embodiments, the TFVF unit includes one or more tangential virus
 filters having a pore size of about 5 nm to about 220 nm, about 210 nm, about 200
 20 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about
 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm,
 about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm,
 about 60 nm, about 55 nm, about 50 nm, about 45 nm, about 40 nm, about 35 nm,
 about 30 nm, about 25 nm, about 20 nm, about 15 nm, or about 10 nm; about 10 nm
 25 to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about
 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm,
 about 110 nm, about 100 nm, about 95 nm, about 90 nm, about 85 nm, about 80 nm,
 about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm,
 about 45 nm, about 40 nm, about 35 nm, about 30 nm, about 25 nm, about 20 nm, or
 30 about 15 nm; about 15 nm to about 220 nm, about 210 nm, about 200 nm, about 190
 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about
 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm, about 90 nm,
 about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm,

about 55 nm, about 50 nm, about 45 nm, about 40 nm, about 35 nm, about 30 nm,
 about 25 nm, or about 20 nm; about 20 nm to about 220 nm, about 210 nm, about 200
 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about
 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm,
 5 about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm,
 about 60 nm, about 55 nm, about 50 nm, about 45 nm, about 40 nm, about 35 nm,
 about 30 nm, or about 25 nm; about 25 nm to about 220 nm, about 210 nm, about 200
 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about
 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm,
 10 about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm,
 about 60 nm, about 55 nm, about 50 nm, about 45 nm, about 40 nm, about 35 nm, or
 about 30 nm; about 30 nm to about 220 nm, about 210 nm, about 200 nm, about 190
 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about
 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm, about 90 nm,
 15 about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm, about 60 nm,
 about 55 nm, about 50 nm, about 45 nm, about 40 nm, or about 35 nm; about 35 nm
 to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about
 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm,
 about 110 nm, about 100 nm, about 95 nm, about 90 nm, about 85 nm, about 80 nm,
 20 about 75 nm, about 70 nm, about 65 nm, about 60 nm, about 55 nm, about 50 nm,
 about 45 nm, or about 40 nm; about 45 nm to about 220 nm, about 210 nm, about 200
 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about
 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm,
 about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, about 65 nm,
 25 about 60 nm, about 55 nm, or about 50 nm; about 50 nm to about 220 nm, about 210
 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about
 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm,
 about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm,
 about 65 nm, about 60 nm, or about 55 nm; about 55 nm to about 220 nm, about 210
 30 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about
 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm,
 about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm,
 about 65 nm, or about 60 nm; about 60 nm to about 220 nm, about 210 nm, about 200

nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, about 70 nm, or about 65 nm; about 65 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm, about 90 nm, about 85 nm, about 80 nm, about 75 nm, or about 70 nm; about 70 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm, about 90 nm, about 85 nm, about 80 nm, or about 75 nm; about 75 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm, about 90 nm, about 85 nm, or about 80 nm; about 80 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, about 100 nm, about 95 nm, or about 90 nm; about 90 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm, about 110 nm, or about 100 nm; about 100 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, about 120 nm, or about 110 nm; about 110 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, about 130 nm, or about 120 nm; about 120 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, about 140 nm, or about 130 nm; about 130 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, about 150 nm, or about 140 nm; about 140 nm to about 220 nm, about 210 nm, about

200 nm, about 190 nm, about 180 nm, about 170 nm, about 160 nm, or about 150 nm; about 150 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, about 170 nm, or about 160 nm; about 160 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, about 180 nm, or about 170 nm; about 170 nm to about 220 nm, about 210 nm, about 200 nm, about 190 nm, or about 180 nm; about 180 nm to about 220 nm, about 210 nm, about 200 nm, or about 190 nm; about 190 nm to about 220 nm, about 210 nm, or about 200 nm; about 200 nm to about 220 nm or about 210 nm; or about 210 nm to about 220 nm. A variety of virus filters that can be used in a TFVF unit are known in the art. For example, virus filters that can be used in a TFVF unit are commercially available from Asahi Kasei (Tokyo, Japan). In some embodiments, the virus filter is a Planova™ filter (e.g., a Planova™ 15N, a Planova™ 20N, a Planova™ 35N, or a Planova™ 75N). In some embodiments, the virus filter is a Planova™ BioEX filter. The virus filters that can be used in a TFVF unit can include, e.g., hollow fiber membranes.

The connecting conduit and the conduit in the second circuit system can be simple tubing, e.g., biocompatible tubing. Non-limiting examples of useful tubing include silicone rubber, polycarbonate, polypropylene, polyurethane, polydioxanone (PDO), polyhydroxyalkanoate, polyhydroxybutyrate, poly(glycerol sebacate), polyglycolide, polylactide, polycaprolactone, or polyanhydride, or copolymers or derivatives including these and/or other polymers. Alternatively or in addition, any of the conduits described herein can include polyvinyl chloride. Any of the conduits described herein can be, e.g., weldable transfer tubing.

In some embodiments, the internal diameter of the connecting conduit and/or the conduit in the second circuit system can be about 5 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, about 32 mm, about 30 mm, about 28 mm, about 26 mm, about 24 mm, about 22 mm, about 20 mm, about 18 mm, about 16 mm, about 14 mm, about 12 mm, about 10 mm, about 8 mm, or about 6 mm; about 6 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, about 32 mm, about 30 mm,

about 28 mm, about 26 mm, about 24 mm, about 22 mm, about 20 mm, about 18 mm,
 about 16 mm, about 14 mm, about 12 mm, about 10 mm, or about 8 mm; about 8 mM
 to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75
 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48
 5 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36
 mm, about 34 mm, about 32 mm, about 30 mm, about 28 mm, about 26 mm, about 24
 mm, about 22 mm, about 20 mm, about 18 mm, about 16 mm, about 14 mm, about 12
 mm, or about 10 mm; about 10 mm to about 100 mm, about 95 mm, about 90 mm,
 about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm,
 10 about 55 mm, about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm,
 about 40 mm, about 38 mm, about 36 mm, about 34 mm, about 32 mm, about 30 mm,
 about 28 mm, about 26 mm, about 24 mm, about 22 mm, about 20 mm, about 18 mm,
 about 16 mm, about 14 mm, or about 12 mm; about 12 mm to about 100 mm, about
 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about
 15 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm, about
 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, about
 32 mm, about 30 mm, about 28 mm, about 26 mm, about 24 mm, about 22 mm, about
 20 mm, about 18 mm, about 16 mm, or about 14 mm; about 14 mm to about 100 mm,
 about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm,
 20 about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm,
 about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm,
 about 32 mm, about 30 mm, about 28 mm, about 26 mm, about 24 mm, about 22 mm,
 about 20 mm, about 18 mm, or about 16 mm; about 16 mm to about 100 mm, about
 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about
 25 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm, about
 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, about
 32 mm, about 30 mm, about 28 mm, about 26 mm, about 24 mm, about 22 mm, about
 20 mm, or about 18 mm; about 18 mm to about 100 mm, about 95 mm, about 90 mm,
 about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm,
 30 about 55 mm, about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm,
 about 40 mm, about 38 mm, about 36 mm, about 34 mm, about 32 mm, about 30 mm,
 about 28 mm, about 26 mm, about 24 mm, about 22 mm, or about 20 mm; about 20
 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about

75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about
 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about
 36 mm, about 34 mm, about 32 mm, about 30 mm, about 28 mm, about 26 mm, about
 24 mm, or about 22 mm; about 22 mm to about 100 mm, about 95 mm, about 90 mm,
 5 about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm,
 about 55 mm, about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm,
 about 40 mm, about 38 mm, about 36 mm, about 34 mm, about 32 mm, about 30 mm,
 about 28 mm, about 26 mm, or about 24 mm; about 24 mm to about 100 mm, about
 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about
 10 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm, about
 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm, about
 32 mm, about 30 mm, about 28 mm, or about 26 mm; about 26 mm to about 50 mm,
 about 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm,
 about 36 mm, about 34 mm, about 32 mm, about 30 mm, or about 28 mm; about 28
 15 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about
 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about
 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, about 38 mm, about
 36 mm, about 34 mm, about 32 mm, or about 30 mm; about 30 mm to about 100 mm,
 about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm,
 20 about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm,
 about 44 mm, about 42 mm, about 40 mm, about 38 mm, about 36 mm, about 34 mm,
 or about 32 mm; about 32 mm to about 100 mm, about 95 mm, about 90 mm, about
 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about
 55 mm, about 50 mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, about
 25 40 mm, about 38 mm, about 36 mm, or about 34 mm; about 34 mm to about 100 mm,
 about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm,
 about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm,
 about 44 mm, about 42 mm, about 40 mm, about 38 mm, or about 36 mm; about 36
 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about
 30 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about
 48 mm, about 46 mm, about 44 mm, about 42 mm, about 40 mm, to about 38 mm;
 about 38 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80
 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50

mm, about 48 mm, about 46 mm, about 44 mm, about 42 mm, or about 40 mm; about 40 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, about 46 mm, about 44 mm, or about 42 mm; about 42 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, or about 46 mm; about 46 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, about 48 mm, or about 46 mm; about 48 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, about 55 mm, about 50 mm, or about 48 mm; about 50 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, about 60 mm, or about 55 mm; about 55 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, about 65 mm, or about 60 mm; about 60 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, about 70 mm, or about 65 mm; about 65 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, about 75 mm, or about 70 mm; about 70 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, about 80 mm, or about 75 mm; about 75 mm to about 100 mm, about 95 mm, about 90 mm, about 85 mm, or about 80 mm; about 80 mm to about 100 mm, about 95 mm, about 90 mm, or about 85 mm; about 85 mm to about 100 mm, about 95 mm, or about 90 mm; about 90 mm to about 100 mm or about 95 mm; or about 95 mm to about 100 mm.

In some embodiments, the connecting conduit and/or the conduit in the second circuit system has a total length of about 1.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, about 10 cm, about 8.0 cm, about 6.0 cm, about 4.0 cm, or about 2.0 cm; about 2.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm,

about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, about 10 cm,
 about 8.0 cm, about 6.0 cm, or about 4.0 cm; about 4.0 cm to about 75 cm, about 70
 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm,
 about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, about 16 cm,
 5 about 14 cm, about 12 cm, about 10 cm, about 8.0 cm, or about 6.0 cm; about 6.0 cm
 to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm,
 about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm,
 about 18 cm, about 16 cm, about 14 cm, about 12 cm, about 10 cm, or about 8.0 cm;
 about 8.0 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm,
 10 about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm,
 about 20 cm, about 18 cm, about 16 cm, about 14 cm, about 12 cm, or about 10 cm;
 about 10 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm,
 about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm,
 about 20 cm, about 18 cm, about 16 cm, about 14 cm, or about 12 cm; about 12 cm to
 15 about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, about 50 cm,
 about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm, about 20 cm,
 about 18 cm, about 16 cm, or about 14 cm; about 14 cm to about 75 cm, about 70 cm,
 about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm,
 about 35 cm, about 30 cm, about 25 cm, about 20 cm, about 18 cm, or about 16 cm;
 20 about 16 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm,
 about 50 cm, about 45 cm, about 40 cm, about 35 cm, about 30 cm, about 25 cm,
 about 20 cm, or about 18 cm; about 18 cm to about 75 cm, about 70 cm, about 65 cm,
 about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, about 35 cm,
 about 30 cm, about 25 cm, or about 20 cm; about 20 cm to about 75 cm, about 70 cm,
 25 about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm,
 about 35 cm, about 30 cm, or about 25 cm; about 25 cm to about 75 cm, about 70 cm,
 about 65 cm, about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm,
 about 35 cm, or about 30 cm; about 30 cm to about 75 cm, about 70 cm, about 65 cm,
 about 60 cm, about 55 cm, about 50 cm, about 45 cm, about 40 cm, or about 35 cm;
 30 about 35 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm,
 about 50 cm, about 45 cm, or about 40 cm; about 40 cm to about 75 cm, about 70 cm,
 about 65 cm, about 60 cm, about 55 cm, about 50 cm, or about 45 cm; about 45 cm to
 about 75 cm, about 70 cm, about 65 cm, about 60 cm, about 55 cm, or about 50 cm;

about 50 cm to about 75 cm, about 70 cm, about 65 cm, about 60 cm, or about 55 cm; about 55 cm to about 75 cm, about 70 cm, about 65 cm, or about 60 cm; about 60 cm to about 75 cm, about 70 cm, or about 65 cm; about 65 cm to about 75 cm or about 70 cm; or about 70 cm to about 75 cm.

5 Additional examples of conduits and properties of conduits that can be used in the second circuit system are well-known by those in the art.

In some examples, the second circuit system includes one, two, three, four, or five ports. In some examples, the at least one port in the second circuit system can be a valve. In other examples, the at least one port in the second circuit system can be an injection port or can have a ribbed threading. The at least one port in the second circuit system can be any type of port commonly known in the art.

In some examples, the second circuit system can further include a pump (e.g., a low-turbulence pump, e.g., a peristaltic pump) disposed in the connecting conduit and/or the conduit in second system. In some embodiments, the second circuit system does not include a holding tank. In some embodiments, the connecting conduit is not fluidly connected to a holding tank.

In some examples, step (f) includes continuously flowing (e.g., unidirectionally or bidirectionally flowing) the collected fluid of step (d) into the second system (using the connecting conduit) at a rate of about 0.05 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute, about 20 mL/minute, about 18 mL/minute, about 16 mL/minute, about 14 mL/minute, about 12 mL/minute, about 10 mL/minute, about 9.5 mL/minute, about 9.0 mL/minute, about 8.5 mL/minute, about 8.0 mL/minute, about 7.5 mL/minute, about 7.0 mL/minute, about 6.5 mL/minute, about 6.0 mL/minute, about 5.5 mL/minute, about 5.0 mL/minute, about 4.5 mL/minute, about 4.0 mL/minute, about 3.5

mL/minute, about 3.0 mL/minute, about 2.5 mL/minute, about 2.0 mL/minute, about
 1.5 mL/minute, about 1.0 mL/minute, about 0.5 mL/minute, or about 0.1 mL/minute;
 about 0.1 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute,
 about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about
 5 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about
 800 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute,
 about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450
 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute,
 10 about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100
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 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 about 50 mL/minute, about 25 mL/minute, about 24 mL/minute, about 22 mL/minute,
 25 or about 20 mL/minute; about 20 mL/minute to about 100 L/minute, about 90
 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50
 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute,
 30 about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
 mL/minute, about 150 mL/minute, about 100 mL/minute, about 50 mL/minute, about

25 mL/minute, about 24 mL/minute, or about 22 mL/minute; about 22 mL/minute to
 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about
 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 5 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 10 about 50 mL/minute, about 25 mL/minute or about 24 mL/minute; about 23
 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70
 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800
 15 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute,
 about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450
 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute,
 about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100
 mL/minute, about 50 mL/minute, or about 25 mL/minute; about 25 mL/minute to
 20 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about
 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 25 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, or
 about 50 mL/minute; about 50 mL/minute to about 100 L/minute, about 90 L/minute,
 about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about
 30 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850
 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute,
 about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500

mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute,
 about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150
 mL/minute, or about 100 mL/minute; about 100 mL/minute to about 100 L/minute,
 about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about
 5 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute,
 about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 10 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
 mL/minute, or about 150 mL/minute; about 150 mL/minute to about 100 L/minute,
 about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about
 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 15 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute,
 about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, or about 200
 mL/minute; about 200 mL/minute to about 100 L/minute, about 90 L/minute, about
 20 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40
 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850
 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute,
 about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500
 25 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute,
 about 300 mL/minute, or about 250 mL/minute; about 250 mL/minute to about 100
 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60
 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 30 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, or about 300 mL/minute; about 300

mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70
 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800
 5 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute,
 about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450
 mL/minute, about 400 mL/minute, or about 350 mL/minute; about 350 mL/minute to
 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about
 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 10 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute, or
 about 400 mL/minute; about 400 mL/minute to about 100 L/minute, about 90
 15 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50
 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute,
 about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550
 20 mL/minute, about 500 mL/minute, or about 450 mL/minute; about 450 mL/minute to
 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about
 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 25 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, or about 500 mL/minute; about 500 mL/minute to
 about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about
 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 30 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, or about 550 mL/minute; about 550 mL/minute to about 100 L/minute,
 about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about

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L/minute, about 5 L/minute, about 1 L/minute, or about 950 mL/minute; about 950 mL/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, or about 1 L/minute; about 1 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, about 10 L/minute, or about 5 L/minute; about 5 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, about 20 L/minute, or about 10 L/minute; about 10 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, about 30 L/minute, or about 20 L/minute; about 20 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, about 40 L/minute, or about 30 L/minute; about 30 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, or about 40 L/minute; about 40 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, about 50 L/minute, or about 30 L/minute; about 50 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, about 60 L/minute, or about 50 L/minute; about 60 L/minute to about 100 L/minute, about 90 L/minute, about 80 L/minute, about 70 L/minute, or about 60 L/minute; about 70 L/minute to about 100 L/minute, about 90 L/minute, or about 80 L/minute; about 80 L/minute to about 100 L/minute or about 90 L/minute; or about 90 L/minute to about 100 L/minute.

In some examples, the filtrate collected from the TFVF unit in step (f) includes a concentration of recombinant therapeutic protein of about 0.1 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34

mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL,
 about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL,
 about 7 mg/mL, about 6 mg/mL, about 5 mg/mL, about 4 mg/mL, about 3 mg/mL,
 5 about 2.5 mg/mL, about 2.0 mg/mL, about 1.5 mg/mL, about 1.0 mg/mL, or about 0.5
 mg/mL; about 0.5 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about
 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL,
 about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76
 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about
 10 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL,
 about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48
 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about
 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL,
 about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20
 15 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about
 10 mg/mL, about 9 mg/mL, about 8 mg/mL, about 7 mg/mL, about 6 mg/mL, about 5
 mg/mL, about 4 mg/mL, about 3 mg/mL, about 2.5 mg/mL, about 2.0 mg/mL, about
 1.5 mg/mL, or about 1.0 mg/mL; about 1.0 mg/mL to about 100 mg/mL, about 98
 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 20 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
 25 about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about
 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL,
 about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL, about 7 mg/mL,
 about 6 mg/mL, about 5 mg/mL, about 4 mg/mL, about 3 mg/mL, about 2.5 mg/mL,
 30 about 2.0 mg/mL, or about 1.5 mg/mL; about 1.5 mg/mL to about 100 mg/mL, about
 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88
 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,

about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
5 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about
22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL,
about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL, about 7 mg/mL,
about 6 mg/mL, about 5 mg/mL, about 4 mg/mL, about 3 mg/mL, about 2.5 mg/mL,
or about 2.0 mg/mL; about 2.0 mg/mL to about 100 mg/mL, about 98 mg/mL, 96
10 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about
86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL,
about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68
mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about
58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL,
15 about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40
mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about
30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL,
about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12
mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL, about 7 mg/mL, about 6
20 mg/mL, about 5 mg/mL, about 4 mg/mL, about 3 mg/mL, or about 2.5 mg/mL; about
2.5 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL,
about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84
mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about
74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL,
25 about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56
mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about
46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL,
about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28
mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about
30 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10 mg/mL,
about 9 mg/mL, about 8 mg/mL, about 7 mg/mL, about 6 mg/mL, about 5 mg/mL,
about 4 mg/mL, or about 3 mg/mL; about 3 mg/mL to about 100 mg/mL, about 98
mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88

mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
 about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about
 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL,
 about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL, about 7 mg/mL,
 about 6 mg/mL, about 5 mg/mL, or about 4 mg/mL; about 4 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL,
 about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL,
 about 7 mg/mL, about 6 mg/mL, or about 5 mg/mL; about 5 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL,
 about 14 mg/mL, about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL,
 about 7 mg/mL, or about 6 mg/mL; about 6 mg/mL to about 100 mg/mL, about 98
 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88

mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about
 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL,
 about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60
 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about
 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL,
 about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32
 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about
 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about 14 mg/mL,
 about 12 mg/mL, about 10 mg/mL, about 9 mg/mL, about 8 mg/mL, or about 7
 mg/mL; about 7 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94
 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about
 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL,
 about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66
 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about
 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL,
 about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38
 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about
 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL,
 about 18 mg/mL, about 16 mg/mL, about 14 mg/mL, about 12 mg/mL, about 10
 mg/mL, about 9 mg/mL, or about 8 mg/mL; about 8 mg/mL to about 100 mg/mL,
 about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL,
 about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80
 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about
 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL,
 about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52
 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about
 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL,
 about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24
 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, about
 14 mg/mL, about 12 mg/mL, about 10 mg/mL, or about 9 mg/mL; about 9 mg/mL to
 about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL,
 about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82
 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about

72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL,
 about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54
 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about
 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL,
 5 about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26
 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about
 16 mg/mL, about 14 mg/mL, about 12 mg/mL, or about 10 mg/mL; about 10 mg/mL
 to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
 10 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL,
 about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64
 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about
 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL,
 about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36
 15 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about
 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL,
 about 16 mg/mL, about 14 mg/mL, or about 12 mg/mL; about 12 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 20 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 25 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, about 18 mg/mL, about 16 mg/mL, or
 about 14 mg/mL; about 14 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,
 about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86
 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about
 30 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL,
 about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58
 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about
 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL,

about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30
 mg/mL, about 28 mg/mL, about 26 mg/mL, about 24 mg/mL, about 22 mg/mL, about
 20 mg/mL, about 18 mg/mL, or about 16 mg/mL; about 16 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 5 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 10 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, about
 24 mg/mL, about 22 mg/mL, about 20 mg/mL, or about 18 mg/mL; about 18 mg/mL
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 15 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL,
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 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about
 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL,
 about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36
 20 mg/mL, about 34 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about
 26 mg/mL, about 24 mg/mL, about 22 mg/mL, or about 20 mg/mL; about 20 mg/mL
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 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL,
 25 about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64
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 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,

about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
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 5 mg/mL, about 32 mg/mL, about 30 mg/mL, about 28 mg/mL, about 26 mg/mL, or
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 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL,
 10 about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58
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 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL,
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 mg/mL, about 28 mg/mL, or about 26 mg/mL; about 26 mg/mL to about 100 mg/mL,
 15 about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL,
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 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
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 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34
 30 mg/mL, about 32 mg/mL, or about 30 mg/mL; about 30 mg/mL to about 100 mg/mL,
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 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about

70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL,
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 42 mg/mL, about 40 mg/mL, about 38 mg/mL, about 36 mg/mL, about 34 mg/mL, or
 5 about 32 mg/mL; about 32 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,
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 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL,
 about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58
 10 mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about
 48 mg/mL, about 46 mg/mL, about 44 mg/mL, about 42 mg/mL, about 40 mg/mL,
 about 38 mg/mL, about 36 mg/mL, or about 34 mg/mL; about 34 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
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 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL,
 about 42 mg/mL, about 40 mg/mL, about 38 mg/mL, or about 36 mg/mL; about 36
 20 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
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 25 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL,
 about 44 mg/mL, about 42 mg/mL, about 40 mg/mL, or about 38 mg/mL; about 38
 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL,
 30 about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64
 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about
 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL,
 about 44 mg/mL, about 42 mg/mL, or about 40 mg/mL; about 40 mg/mL to about 100

mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
5 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
52 mg/mL, about 50 mg/mL, about 48 mg/mL, about 46 mg/mL, about 44 mg/mL, or
about 42 mg/mL; about 42 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,
about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86
mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about
10 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL,
about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58
mg/mL, about 56 mg/mL, about 54 mg/mL, about 52 mg/mL, about 50 mg/mL, about
48 mg/mL, about 46 mg/mL, or about 44 mg/mL; about 44 mg/mL to about 100
mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
15 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, about
52 mg/mL, about 50 mg/mL, about 48 mg/mL, or about 46 mg/mL; about 46 mg/mL
20 to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL,
about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64
mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about
25 54 mg/mL, about 52 mg/mL, about 50 mg/mL, or about 48 mg/mL; about 48 mg/mL
to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92
mg/mL, about 90 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about
82 mg/mL, about 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL,
about 72 mg/mL, about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64
30 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about
54 mg/mL, about 52 mg/mL, or about 50 mg/mL; about 50 mg/mL to about 100
mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about

80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, about 58 mg/mL, about 56 mg/mL, about 54 mg/mL, or
 about 52 mg/mL; about 52 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,
 5 about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86
 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about
 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL,
 about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, about 60 mg/mL, about 58
 mg/mL, about 56 mg/mL, or about 54 mg/mL; about 54 mg/mL to about 100 mg/mL,
 10 about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL,
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 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about
 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL,
 about 60 mg/mL, about 58 mg/mL, or about 56 mg/mL; about 56 mg/mL to about 100
 15 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62
 mg/mL, about 60 mg/mL, or about 58 mg/mL; about 58 mg/mL to about 100 mg/mL,
 20 about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90 mg/mL,
 about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80
 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about
 70 mg/mL, about 68 mg/mL, about 66 mg/mL, about 64 mg/mL, about 62 mg/mL, or
 about 60 mg/mL; about 60 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL,
 25 about 94 mg/mL, about 92 mg/mL, about 90 mg/mL, about 88 mg/mL, about 86
 mg/mL, about 84 mg/mL, about 82 mg/mL, about 80 mg/mL, about 78 mg/mL, about
 76 mg/mL, about 74 mg/mL, about 72 mg/mL, about 70 mg/mL, about 68 mg/mL,
 about 66 mg/mL, about 64 mg/mL, or about 62 mg/mL; about 62 mg/mL to about 100
 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92 mg/mL, about 90
 30 mg/mL, about 88 mg/mL, about 86 mg/mL, about 84 mg/mL, about 82 mg/mL, about
 80 mg/mL, about 78 mg/mL, about 76 mg/mL, about 74 mg/mL, about 72 mg/mL,
 about 70 mg/mL, about 68 mg/mL, about 66 mg/mL, or about 64 mg/mL; about 64
 mg/mL to about 100 mg/mL, about 98 mg/mL, 96 mg/mL, about 94 mg/mL, about 92

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In some examples of these methods, the third period of time can be about 0.1 minute to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, about 15 minutes, about 10 minutes, about 5 minutes, about 2 minutes, or about 1 minute; about 1 minute to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, about 15 minutes, about 10 minutes, about 5 minutes, or about 2 minutes; about 2 minutes to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, about 15 minutes, about 10 minutes, or about 5 minutes; about 5 minutes to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0

hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55
 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
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 minutes; about 10 minutes to about 12 hours, about 11.5 hours, about 11 hours, about
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 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
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 minutes to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about
 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5
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 about 25 minutes, or about 20 minutes; about 20 minutes to about 12 hours, about
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 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5
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 hours, about 1.0 hour, about 55 minutes, about 50 minutes, about 45 minutes, about
 40 minutes, about 35 minutes, about 30 minutes, or about 25 minutes; about 25
 minutes to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about
 25 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5
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 30 about 30 minutes to about 12 hours, about 11.5 hours, about 11 hours, about 10.5
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 minutes, about 50 minutes, about 45 minutes, about 40 minutes, or about 35 minutes;
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 minutes, about 50 minutes, about 45 minutes, or about 40 minutes; about 40 minutes
 to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours,
 10 about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours,
 about 7 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours,
 about 4.5 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours,
 about 2.0 hours, about 1.5 hours, about 1.0 hour, about 55 minutes, about 50 minutes,
 or about 45 minutes; about 45 minutes to about 12 hours, about 11.5 hours, about 11
 15 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5
 hours, about 8.0 hours, about 7.5 hours, about 7 hours, about 6.5 hours, about 6.0
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 hour, about 55 minutes, or about 50 minutes; about 50 minutes to about 12 hours,
 20 about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours,
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 about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours,
 about 1.5 hours, about 1.0 hour, or about 55 minutes; about 55 minutes to about 12
 25 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5
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 hours, about 6.5 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5
 hours, about 4.0 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0
 hours, about 1.5 hours, or about 1.0 hour; about 1.0 hour to about 12 hours, about 11.5
 30 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0
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 hours, about 6.0 hours, about 5.5 hours, about 5.0 hours, about 4.5 hours, about 4.0
 hours, about 3.5 hours, about 3.0 hours, about 2.5 hours, about 2.0 hours, or about 1.5

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11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, about 7 hours, or about 6.5 hours; about 6.5 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, about 7.5 hours, or about 7 hours; about 7 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, about 8.0 hours, or about 7.5 hours; about 7.5 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, about 8.5 hours, or about 8.0 hours; about 8.0 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, about 9.0 hours, or about 8.5 hours; about 8.5 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, about 9.5 hours, or about 9.0 hours; about 9.0 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, about 10 hours, or about 9.5 hours; about 9.5 hours to about 12 hours, about 11.5 hours, about 11 hours, about 10.5 hours, or about 10 hours; about 10 hours to about 12 hours, about 11.5 hours, about 11 hours, or about 10.5 hours; about 10.5 hours to about 12 hours, about 11.5 hours, or about 11 hours; about 11 hours to about 12 hours, or about 11.5 hours.

In some examples of these methods, the third period of time can be, e.g., about 12 hours to about 72 hours, about 12 hours to about 69 hours, about 12 hours to about 66 hours, about 12 hours to about 63 hours, about 12 hours to about 60 hours, about 12 hours to about 57 hours, about 12 hours to about 54 hours, about 12 hours to about 51 hours, about 12 hours to about 48 hours, about 12 hours to about 45 hours, about 12 hours to about 42 hours, about 12 hours to about 39 hours, about 12 hours to about 36 hours, about 12 hours to about 33 hours, about 12 hours to about 30 hours, about 12 hours to about 27 hours, about 12 hours to about 24 hours, about 12 hours to about 21 hours, about 12 hours to about 18 hours, about 12 hours to about 15 hours, about 15 hours to about 72 hours, about 15 hours to about 69 hours, about 15 hours to about 66 hours, about 15 hours to about 63 hours, about 15 hours to about 60 hours, about 15 hours to about 57 hours, about 15 hours to about 54 hours, about 15 hours to about 51 hours, about 15 hours to about 48 hours, about 15 hours to about 45 hours, about 15 hours to about 42 hours, about 15 hours to about 39 hours, about 15 hours to about 36 hours, about 15 hours to about 33 hours, about 15 hours to about 30 hours, about

15 hours to about 27 hours, about 15 hours to about 24 hours, about 15 hours to about 21 hours, about 15 hours to about 18 hours, about 18 hours to about 72 hours, about 18 hours to about 69 hours, about 18 hours to about 66 hours, about 18 hours to about 63 hours, about 18 hours to about 60 hours, about 18 hours to about 57 hours, about 5 18 hours to about 54 hours, about 18 hours to about 51 hours, about 18 hours to about 48 hours, about 18 hours to about 45 hours, about 18 hours to about 42 hours, about 18 hours to about 39 hours, about 18 hours to about 36 hours, about 18 hours to about 33 hours, about 18 hours to about 30 hours, about 18 hours to about 27 hours, about 18 hours to about 24 hours, about 18 hours to about 21 hours, about 21 hours to about 10 72 hours, about 21 hours to about 69 hours, about 21 hours to about 66 hours, about 21 hours to about 63 hours, about 21 hours to about 60 hours, about 21 hours to about 57 hours, about 21 hours to about 54 hours, about 21 hours to about 51 hours, about 21 hours to about 48 hours, about 21 hours to about 45 hours, about 21 hours to about 42 hours, about 21 hours to about 39 hours, about 21 hours to about 36 hours, about 15 21 hours to about 33 hours, about 21 hours to about 30 hours, about 21 hours to about 27 hours, about 21 hours to about 24 hours, about 24 hours to about 72 hours, about 24 hours to about 69 hours, about 24 hours to about 66 hours, about 24 hours to about 63 hours, about 24 hours to about 60 hours, about 24 hours to about 57 hours, about 24 hours to about 54 hours, about 24 hours to about 51 hours, about 24 hours to about 20 48 hours, about 24 hours to about 45 hours, about 24 hours to about 42 hours, about 24 hours to about 39 hours, about 24 hours to about 36 hours, about 24 hours to about 33 hours, about 24 hours to about 30 hours, about 24 hours to about 27 hours, about 27 hours to about 72 hours, about 27 hours to about 69 hours, about 27 hours to about 66 hours, about 27 hours to about 63 hours, about 27 hours to about 60 hours, about 25 27 hours to about 57 hours, about 27 hours to about 54 hours, about 27 hours to about 51 hours, about 27 hours to about 48 hours, about 27 hours to about 45 hours, about 27 hours to about 42 hours, about 27 hours to about 39 hours, about 27 hours to about 36 hours, about 27 hours to about 33 hours, about 27 hours to about 30 hours, about 30 hours to about 72 hours, about 30 hours to about 69 hours, about 30 hours to about 30 66 hours, about 30 hours to about 63 hours, about 30 hours to about 60 hours, about 30 hours to about 57 hours, about 30 hours to about 54 hours, about 30 hours to about 51 hours, about 30 hours to about 48 hours, about 30 hours to about 45 hours, about 30 hours to about 42 hours, about 30 hours to about 39 hours, about 30 hours to about

36 hours, about 30 hours to about 33 hours, about 33 hours to about 72 hours, about
33 hours to about 69 hours, about 33 hours to about 66 hours, about 33 hours to about
63 hours, about 33 hours to about 60 hours, about 33 hours to about 57 hours, about
33 hours to about 54 hours, about 33 hours to about 51 hours, about 33 hours to about
5 48 hours, about 33 hours to about 45 hours, about 33 hours to about 42 hours, about
33 hours to about 39 hours, about 33 hours to about 36 hours, about 36 hours to about
72 hours, about 36 hours to about 69 hours, about 36 hours to about 66 hours, about
36 hours to about 63 hours, about 36 hours to about 60 hours, about 36 hours to about
57 hours, about 36 hours to about 54 hours, about 36 hours to about 51 hours, about
10 36 hours to about 48 hours, about 36 hours to about 45 hours, about 36 hours to about
42 hours, about 36 hours to about 39 hours, about 39 hours to about 72 hours, about
39 hours to about 69 hours, about 39 hours to about 66 hours, about 39 hours to about
63 hours, about 39 hours to about 60 hours, about 39 hours to about 57 hours, about
39 hours to about 54 hours, about 39 hours to about 51 hours, about 39 hours to about
15 48 hours, about 39 hours to about 45 hours, about 39 hours to about 42 hours, about
42 hours to about 72 hours, about 42 hours to about 69 hours, about 42 hours to about
66 hours, about 42 hours to about 63 hours, about 42 hours to about 60 hours, about
42 hours to about 57 hours, about 42 hours to about 54 hours, about 42 hours to about
51 hours, about 42 hours to about 48 hours, about 42 hours to about 45 hours, about
20 45 hours to about 72 hours, about 45 hours to about 69 hours, about 45 hours to about
66 hours, about 45 hours to about 63 hours, about 45 hours to about 60 hours, about
45 hours to about 57 hours, about 45 hours to about 54 hours, about 45 hours to about
51 hours, about 45 hours to about 48 hours, about 48 hours to about 72 hours, about
48 hours to about 69 hours, about 48 hours to about 66 hours, about 48 hours to about
25 63 hours, about 48 hours to about 60 hours, about 48 hours to about 57 hours, about
48 hours to about 54 hours, about 48 hours to about 51 hours, about 51 hours to about
72 hours, about 51 hours to about 69 hours, about 51 hours to about 66 hours, about
51 hours to about 63 hours, about 51 hours to about 60 hours, about 51 hours to about
57 hours, about 51 hours to about 54 hours, about 54 hours to about 72 hours, about
30 54 hours to about 69 hours, about 54 hours to about 66 hours, about 54 hours to about
63 hours, about 54 hours to about 60 hours, about 54 hours to about 57 hours, about
57 hours to about 72 hours, about 57 hours to about 69 hours, about 57 hours to about
66 hours, about 57 hours to about 63 hours, about 57 hours to about 60 hours, about

In some embodiments of these methods, the collected fluid of step (d) that is continuously flowed into the second circuit system including the TFVF unit can include a total virus titer of about 10^{-1} to about 10^{-20} , about 10^{-1} to about 10^{-19} , about 10^{-1} to about 10^{-18} , about 10^{-1} to about 10^{-17} , about 10^{-1} to about 10^{-16} , about 10^{-1} to about 10^{-15} , about 10^{-1} to about 10^{-14} , about 10^{-1} to about 10^{-13} , about 10^{-1} to about 10^{-12} , about 10^{-1} to about 10^{-11} , about 10^{-1} to about 10^{-10} , about 10^{-1} to about 10^{-9} , about 10^{-1} to about 10^{-8} , about 10^{-1} to about 10^{-7} , about 10^{-1} to about 10^{-6} , about 10^{-1} to about 10^{-5} , about 10^{-1} to about 10^{-4} , about 10^{-1} to about 10^{-3} , about 10^{-1} to about 10^{-2} , about 10^{-2} to about 10^{-20} , about 10^{-2} to about 10^{-19} , about 10^{-2} to about 10^{-18} , about 10^{-2} to about 10^{-17} , about 10^{-2} to about 10^{-16} , about 10^{-2} to about 10^{-15} , about 10^{-2} to about 10^{-14} , about 10^{-2} to about 10^{-13} , about 10^{-2} to about 10^{-12} , about 10^{-2} to about 10^{-11} , about 10^{-2} to about 10^{-10} , about 10^{-2} to about 10^{-9} , about 10^{-2} to about 10^{-8} , about 10^{-2} to about 10^{-7} , about 10^{-2} to about 10^{-6} , about 10^{-2} to about 10^{-5} , about 10^{-2} to about 10^{-4} , about 10^{-2} to about 10^{-3} , about 10^{-3} to about 10^{-20} , about 10^{-3} to about 10^{-19} , about 10^{-3} to about 10^{-18} , about 10^{-3} to about 10^{-17} , about 10^{-3} to about 10^{-16} , about 10^{-3} to about 10^{-15} , about 10^{-3} to about 10^{-14} , about 10^{-3} to about 10^{-13} , about 10^{-3} to about 10^{-12} , about 10^{-3} to about 10^{-11} , about 10^{-3} to about 10^{-10} , about 10^{-3} to about 10^{-9} , about 10^{-3} to about 10^{-8} , about 10^{-3} to about 10^{-7} , about 10^{-3} to about 10^{-6} , about 10^{-3} to about 10^{-5} , about 10^{-3} to about 10^{-4} , about 10^{-4} to about 10^{-20} , about 10^{-4} to about 10^{-19} , about 10^{-4} to about 10^{-18} , about 10^{-4} to about 10^{-17} , about 10^{-4} to about 10^{-16} , about 10^{-4} to about 10^{-15} , about 10^{-4} to about 10^{-14} , about 10^{-4} to about 10^{-13} , about 10^{-4} to about 10^{-12} , about 10^{-4} to about 10^{-11} , about 10^{-4} to about 10^{-10} , about 10^{-4} to about 10^{-9} , about 10^{-4} to about 10^{-8} , about 10^{-4} to about 10^{-7} , about 10^{-4} to about 10^{-6} , about 10^{-4} to about 10^{-5} , about 10^{-5} to about 10^{-20} , about 10^{-5} to about 10^{-19} , about 10^{-5} to about 10^{-18} , about 10^{-5} to about 10^{-17} , about 10^{-5} to about 10^{-16} , about 10^{-5} to about 10^{-15} , about 10^{-5} to about 10^{-14} , about 10^{-5} to about 10^{-13} , about 10^{-5} to about 10^{-12} , about 10^{-5} to about 10^{-11} , about 10^{-5} to about 10^{-10} , about 10^{-5} to about 10^{-9} , about 10^{-5} to about 10^{-8} , about 10^{-5} to about 10^{-7} , about 10^{-5} to about 10^{-6} , about 10^{-6} to about 10^{-20} , about 10^{-6} to about 10^{-19} , about 10^{-6} to about 10^{-18} , about 10^{-6} to about 10^{-17} , about 10^{-6} to about 10^{-16} , about 10^{-6} to about 10^{-15} , about 10^{-6} to about 10^{-14} , about 10^{-6} to about 10^{-13} , about 10^{-6} to about 10^{-12} , about 10^{-6} to about 10^{-11} , about 10^{-6} to about 10^{-10} , about 10^{-6} to about 10^{-9} , about 10^{-6} to about 10^{-8} , about 10^{-6} to about 10^{-7} , about 10^{-6} to about 10^{-6} .

¹⁶, about 10^{-6} to about 10^{-15} , about 10^{-6} to about 10^{-14} , about 10^{-6} to about 10^{-13} , about 10^{-6} to about 10^{-12} , about 10^{-6} to about 10^{-11} , about 10^{-6} to about 10^{-10} , about 10^{-6} to about 10^{-9} , about 10^{-6} to about 10^{-8} , about 10^{-6} to about 10^{-7} , about 10^{-7} to about 10^{-20} , about 10^{-7} to about 10^{-19} , about 10^{-7} to about 10^{-18} , about 10^{-7} to about 10^{-17} , about 10^{-7} to about 10^{-16} , about 10^{-7} to about 10^{-15} , about 10^{-7} to about 10^{-14} , about 10^{-7} to about 10^{-13} , about 10^{-7} to about 10^{-12} , about 10^{-7} to about 10^{-11} , about 10^{-7} to about 10^{-10} , about 10^{-7} to about 10^{-9} , about 10^{-7} to about 10^{-8} , about 10^{-8} to about 10^{-20} , about 10^{-8} to about 10^{-19} , about 10^{-8} to about 10^{-18} , about 10^{-8} to about 10^{-17} , about 10^{-8} to about 10^{-16} , about 10^{-8} to about 10^{-15} , about 10^{-8} to about 10^{-14} , about 10^{-8} to about 10^{-13} , about 10^{-8} to about 10^{-12} , about 10^{-8} to about 10^{-11} , about 10^{-8} to about 10^{-10} , about 10^{-8} to about 10^{-9} , about 10^{-9} to about 10^{-20} , about 10^{-9} to about 10^{-19} , about 10^{-9} to about 10^{-18} , about 10^{-9} to about 10^{-17} , about 10^{-9} to about 10^{-16} , about 10^{-9} to about 10^{-15} , about 10^{-9} to about 10^{-14} , about 10^{-9} to about 10^{-13} , about 10^{-9} to about 10^{-12} , about 10^{-9} to about 10^{-11} , about 10^{-9} to about 10^{-10} , about 10^{-10} to about 10^{-20} , about 10^{-10} to about 10^{-19} , about 10^{-10} to about 10^{-18} , about 10^{-10} to about 10^{-17} , about 10^{-10} to about 10^{-16} , about 10^{-10} to about 10^{-15} , about 10^{-10} to about 10^{-14} , about 10^{-10} to about 10^{-13} , about 10^{-10} to about 10^{-12} , about 10^{-10} to about 10^{-11} , about 10^{-11} to about 10^{-20} , about 10^{-11} to about 10^{-19} , about 10^{-11} to about 10^{-18} , about 10^{-11} to about 10^{-17} , about 10^{-11} to about 10^{-16} , about 10^{-11} to about 10^{-15} , about 10^{-11} to about 10^{-14} , about 10^{-11} to about 10^{-13} , about 10^{-11} to about 10^{-12} , about 10^{-12} to about 10^{-20} , about 10^{-12} to about 10^{-19} , about 10^{-12} to about 10^{-18} , about 10^{-12} to about 10^{-17} , about 10^{-12} to about 10^{-16} , about 10^{-12} to about 10^{-15} , about 10^{-12} to about 10^{-14} , about 10^{-12} to about 10^{-13} , about 10^{-13} to about 10^{-20} , about 10^{-13} to about 10^{-19} , about 10^{-13} to about 10^{-18} , about 10^{-13} to about 10^{-17} , about 10^{-13} to about 10^{-16} , about 10^{-13} to about 10^{-15} , about 10^{-13} to about 10^{-14} , about 10^{-14} to about 10^{-20} , about 10^{-14} to about 10^{-19} , about 10^{-14} to about 10^{-18} , about 10^{-14} to about 10^{-17} , about 10^{-14} to about 10^{-16} , about 10^{-14} to about 10^{-15} , about 10^{-15} to about 10^{-20} , about 10^{-15} to about 10^{-19} , about 10^{-15} to about 10^{-18} , about 10^{-15} to about 10^{-17} , about 10^{-15} to about 10^{-16} , about 10^{-16} to about 10^{-20} , about 10^{-16} to about 10^{-19} , about 10^{-16} to about 10^{-18} , about 10^{-16} to about 10^{-17} , about 10^{-17} to about 10^{-20} , about 10^{-17} to about 10^{-19} , about 10^{-17} to about 10^{-18} , about 10^{-18} to about 10^{-20} , about 10^{-18} to about 10^{-19} , or about 10^{-19} to about 10^{-20} .

In some embodiments, the step of continuously flowing the collected fluid of step (d) into the second circuit system including a TFVF unit can be performed such

that the flux across the filter or one or more filters in the TFVF unit is, e.g., about 10 LMH to about 50 LMH, about 10 LMB to about 48 LMH, about 10 LMH to about 46 LMH, about 10 LMH to about 44 LMH, about 10 LMH to about 42 LMH, about 10 LMH to about 40 LMH, about 10 LMH to about 38 LMH, about 10 LMH to about 36 LMH, about 10 LMH to about 34 LMH, about 10 LMH to about 32 LMH, about 10 LMH to about 30 LMH, about 10 LMH to about 28 LMH, about 10 LMH to about 26 LMH, about 10 LMH to about 24 LMH, about 10 LMH to about 22 LMH, about 10 LMH to about 20 LMH, about 10 LMH to about 18 LMH, about 10 LMH to about 16 LMH, about 10 LMH to about 15 LMH, about 15 LMH to about 50 LMH, about 15 LMB to about 48 LMH, about 15 LMH to about 46 LMH, about 15 LMH to about 44 LMH, about 15 LMH to about 42 LMH, about 15 LMH to about 40 LMH, about 15 LMH to about 38 LMH, about 15 LMH to about 36 LMH, about 15 LMH to about 34 LMH, about 15 LMH to about 32 LMH, about 15 LMH to about 30 LMH, about 15 LMH to about 28 LMH, about 15 LMH to about 26 LMH, about 15 LMH to about 24 LMH, about 15 LMH to about 22 LMH, about 15 LMH to about 20 LMH, about 15 LMH to about 18 LMH, about 20 LMH to about 50 LMH, about 20 LMB to about 48 LMH, about 20 LMH to about 46 LMH, about 20 LMH to about 44 LMH, about 20 LMH to about 42 LMH, about 20 LMH to about 40 LMH, about 20 LMH to about 38 LMH, about 20 LMH to about 36 LMH, about 20 LMH to about 34 LMH, about 20 LMH to about 32 LMH, about 20 LMH to about 30 LMH, about 20 LMH to about 28 LMH, about 20 LMH to about 26 LMH, about 20 LMH to about 24 LMH, about 20 LMH to about 22 LMH, about 22 LMH to about 50 LMH, about 22 LMB to about 48 LMH, about 22 LMH to about 46 LMH, about 22 LMH to about 44 LMH, about 22 LMH to about 42 LMH, about 22 LMH to about 40 LMH, about 22 LMH to about 38 LMH, about 22 LMH to about 36 LMH, about 22 LMH to about 34 LMH, about 22 LMH to about 32 LMH, about 22 LMH to about 30 LMH, about 22 LMH to about 28 LMH, about 22 LMH to about 26 LMH, about 22 LMH to about 24 LMH, about 24 LMH to about 50 LMH, about 24 LMB to about 48 LMH, about 24 LMH to about 46 LMH, about 24 LMH to about 44 LMH, about 24 LMH to about 42 LMH, about 24 LMH to about 40 LMH, about 24 LMH to about 38 LMH, about 24 LMH to about 36 LMH, about 24 LMH to about 34 LMH, about 24 LMH to about 32 LMH, about 24 LMH to about 30 LMH, about 24 LMH to about 28 LMH, about 24 LMH to about 26 LMH, about 26 LMH to about 50 LMH, about 26 LMB to about 48 LMH, about 26

LMH to about 46 LMH, about 26 LMH to about 44 LMH, about 26 LMH to about 42
 LMH, about 26 LMH to about 40 LMH, about 26 LMH to about 38 LMH, about 26
 LMH to about 36 LMH, about 26 LMH to about 34 LMH, about 26 LMH to about 32
 LMH, about 26 LMH to about 30 LMH, about 26 LMH to about 28 LMH, about 28
 5 LMH to about 50 LMH, about 28 LMB to about 48 LMH, about 28 LMH to about 46
 LMH, about 28 LMH to about 44 LMH, about 28 LMH to about 42 LMH, about 28
 LMH to about 40 LMH, about 28 LMH to about 38 LMH, about 28 LMH to about 36
 LMH, about 28 LMH to about 34 LMH, about 28 LMH to about 32 LMH, about 28
 LMH to about 30 LMH, about 30 LMH to about 50 LMH, about 30 LMB to about 48
 10 LMH, about 30 LMH to about 46 LMH, about 30 LMH to about 44 LMH, about 30
 LMH to about 42 LMH, about 30 LMH to about 40 LMH, about 30 LMH to about 38
 LMH, about 30 LMH to about 36 LMH, about 30 LMH to about 34 LMH, about 30
 LMH to about 32 LMH, about 32 LMH to about 50 LMH, about 32 LMB to about 48
 LMH, about 32 LMH to about 46 LMH, about 32 LMH to about 44 LMH, about 32
 15 LMH to about 42 LMH, about 32 LMH to about 40 LMH, about 32 LMH to about 38
 LMH, about 32 LMH to about 36 LMH, about 32 LMH to about 34 LMH, about 34
 LMH to about 50 LMH, about 34 LMB to about 48 LMH, about 34 LMH to about 46
 LMH, about 34 LMH to about 44 LMH, about 34 LMH to about 42 LMH, about 34
 LMH to about 40 LMH, about 34 LMH to about 38 LMH, about 34 LMH to about 36
 20 LMH, about 36 LMH to about 50 LMH, about 36 LMB to about 48 LMH, about 36
 LMH to about 46 LMH, about 36 LMH to about 44 LMH, about 36 LMH to about 42
 LMH, about 36 LMH to about 40 LMH, about 36 LMH to about 38 LMH, about 38
 LMH to about 50 LMH, about 38 LMB to about 48 LMH, about 38 LMH to about 46
 LMH, about 38 LMH to about 44 LMH, about 38 LMH to about 42 LMH, about 38
 25 LMH to about 40 LMH, about 40 LMH to about 50 LMH, about 40 LMB to about 48
 LMH, about 40 LMH to about 46 LMH, about 40 LMH to about 44 LMH, about 40
 LMH to about 42 LMH, about 42 LMH to about 50 LMH, about 42 LMB to about 48
 LMH, about 42 LMH to about 46 LMH, about 42 LMH to about 44 LMH, about 44
 LMH to about 50 LMH, about 44 LMB to about 48 LMH, about 44 LMH to about 46
 30 LMH, about 46 LMH to about 50 LMH, about 46 LMB to about 48 LMH, or about 48
 LMH to about 50 LMH.

In some embodiments, the step of continuously flowing the collected fluid
 from step (d) into the second circuit system including a TFVF unit can be performed

such that the pressure across the filter or the one or more filters in the TFVF unit, or the pressure in the second system including the TFVF is, e.g., about 2 psi to about 40 psi, about 2 psi to about 38 psi, about 2 psi to about 36 psi, about 2 psi to about 34 psi, about 2 psi to about 32 psi, about 2 psi to about 30 psi, about 2 psi to about 28 psi, about 2 psi to about 26 psi, about 2 psi to about 24 psi, about 2 psi to about 22 psi, about 2 psi to about 20 psi, about 2 psi to about 18 psi, about 2 psi to about 16 psi, about 2 psi to about 14 psi, about 2 psi to about 12 psi, about 2 psi to about 10 psi, about 2 psi to about 8 psi, about 2 psi to about 6 psi, about 2 psi to about 4 psi, about 4 psi to about 40 psi, about 4 psi to about 38 psi, about 4 psi to about 36 psi, about 4 psi to about 34 psi, about 4 psi to about 32 psi, about 4 psi to about 30 psi, about 4 psi to about 28 psi, about 4 psi to about 26 psi, about 4 psi to about 24 psi, about 4 psi to about 22 psi, about 4 psi to about 20 psi, about 4 psi to about 18 psi, about 4 psi to about 16 psi, about 4 psi to about 14 psi, about 4 psi to about 12 psi, about 4 psi to about 10 psi, about 4 psi to about 8 psi, about 4 psi to about 6 psi, about 6 psi to about 40 psi, about 6 psi to about 38 psi, about 6 psi to about 36 psi, about 6 psi to about 34 psi, about 6 psi to about 32 psi, about 6 psi to about 30 psi, about 6 psi to about 28 psi, about 6 psi to about 26 psi, about 6 psi to about 24 psi, about 6 psi to about 22 psi, about 6 psi to about 20 psi, about 6 psi to about 18 psi, about 6 psi to about 16 psi, about 6 psi to about 14 psi, about 6 psi to about 12 psi, about 6 psi to about 10 psi, about 6 psi to about 8 psi, about 8 psi to about 40 psi, about 8 psi to about 38 psi, about 8 psi to about 36 psi, about 8 psi to about 34 psi, about 8 psi to about 32 psi, about 8 psi to about 30 psi, about 8 psi to about 28 psi, about 8 psi to about 26 psi, about 8 psi to about 24 psi, about 8 psi to about 22 psi, about 8 psi to about 20 psi, about 8 psi to about 18 psi, about 8 psi to about 16 psi, about 8 psi to about 14 psi, about 8 psi to about 12 psi, about 8 psi to about 10 psi, about 10 psi to about 40 psi, about 10 psi to about 38 psi, about 10 psi to about 36 psi, about 10 psi to about 34 psi, about 10 psi to about 32 psi, about 10 psi to about 30 psi, about 10 psi to about 28 psi, about 10 psi to about 26 psi, about 10 psi to about 24 psi, about 10 psi to about 22 psi, about 10 psi to about 20 psi, about 10 psi to about 18 psi, about 10 psi to about 16 psi, about 10 psi to about 14 psi, about 10 psi to about 12 psi, about 12 psi to about 40 psi, about 12 psi to about 38 psi, about 12 psi to about 36 psi, about 12 psi to about 34 psi, about 12 psi to about 32 psi, about 12 psi to about 30 psi, about 12 psi to about 28 psi, about 12 psi to about 26 psi, about 12 psi to about 24 psi, about 12 psi to about 22 psi,

about 12 psi to about 20 psi, about 12 psi to about 18 psi, about 12 psi to about 16 psi,
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about 14 psi to about 30 psi, about 14 psi to about 28 psi, about 14 psi to about 26 psi,
5 about 14 psi to about 24 psi, about 14 psi to about 22 psi, about 14 psi to about 20 psi,
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about 16 psi to about 38 psi, about 16 psi to about 36 psi, about 16 psi to about 34 psi,
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about 16 psi to about 26 psi, about 16 psi to about 24 psi, about 16 psi to about 22 psi,
10 about 16 psi to about 20 psi, about 16 psi to about 18 psi, about 18 psi to about 40 psi,
about 18 psi to about 38 psi, about 18 psi to about 36 psi, about 18 psi to about 34 psi,
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15 about 20 psi to about 36 psi, about 20 psi to about 34 psi, about 20 psi to about 32 psi,
about 20 psi to about 30 psi, about 20 psi to about 28 psi, about 20 psi to about 26 psi,
about 20 psi to about 24 psi, about 20 psi to about 22 psi, about 22 psi to about 40 psi,
about 22 psi to about 38 psi, about 22 psi to about 36 psi, about 22 psi to about 34 psi,
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20 about 22 psi to about 26 psi, about 22 psi to about 24 psi, about 24 psi to about 40 psi,
about 24 psi to about 38 psi, about 24 psi to about 36 psi, about 24 psi to about 34 psi,
about 24 psi to about 32 psi, about 24 psi to about 30 psi, about 24 psi to about 28 psi,
about 24 psi to about 26 psi, about 26 psi to about 40 psi, about 26 psi to about 38 psi,
about 26 psi to about 36 psi, about 26 psi to about 34 psi, about 26 psi to about 32 psi,
25 about 26 psi to about 30 psi, about 26 psi to about 28 psi, about 28 psi to about 40 psi,
about 28 psi to about 38 psi, about 28 psi to about 36 psi, about 28 psi to about 34 psi,
about 28 psi to about 32 psi, about 28 psi to about 30 psi, about 30 psi to about 40 psi,
about 30 psi to about 38 psi, about 30 psi to about 36 psi, about 30 psi to about 34 psi,
about 30 psi to about 32 psi, about 32 psi to about 40 psi, about 32 psi to about 38 psi,
30 about 32 psi to about 36 psi, about 32 psi to about 34 psi, about 34 psi to about 40 psi,
about 34 psi to about 38 psi, about 34 psi to about 36 psi, about 36 psi to about 40 psi,
about 36 psi to about 38 psi, or about 38 psi to about 40 psi.

Methods of Processing a Fluid Including a Recombinant Therapeutic Protein that Include the Use of a Circuit System Including a TFVF Unit

Also provided are methods of processing a fluid including a recombinant therapeutic protein that include: (a) providing a circuit system including (i) a TFVF unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets of the TFVF unit, including at least one port for flowing fluid into or out of, or both, of the system, where the system is configured such that fluid can be flowed through the conduit and the TFVF unit, and filtrate including the recombinant therapeutic protein can be collected from the TFVF unit; (b) continuously flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port, and collecting filtrate including the recombinant therapeutic protein from the TFVF unit for a period of time. As can be appreciated by those skilled in the art, some embodiments of these methods can further include recirculating any fluid including the recombinant therapeutic protein in the conduit of the circuit system including a TFVF unit that did not pass through the one or more tangential virus filters in the TFVF unit (e.g., acts as a buffer when a new volume of a fluid including the recombinant therapeutic protein is flowed into the conduit of the circuit system including a TFVF unit).

In some embodiments, the TFVF unit includes one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve tangential virus filters. In some examples of these methods, the TFVF unit can include one or more tangential virus filter(s) having a surface area of about 0.05 cm² to about 100 m² (or any of the subranges of this range described herein).

In some embodiments, the TFVF unit includes one or more tangential virus filters having a pore size of about 5 nm to about 220 nm (or any of the subranges of this range described herein). A variety of virus filters that can be used in a TFVF unit are known in the art. For example, virus filters that can be used in a TFVF unit are commercially available from Asahi Kasei (Tokyo, Japan). In some embodiments, the virus filter is a Planova™ filter (e.g., a Planova™ 15N, a Planova™ 20N, a Planova™ 35N, a Planova™ 75N). In some embodiments, the virus filter is a Planova™ BioEX filter. The virus filters that can be used in a TFVF unit can include, e.g., hollow fiber membranes.

The conduit can be simple tubing, e.g., biocompatible tubing. Non-limiting examples of useful tubing include silicone rubber, polyurethane, polydioxanone (PDO), polyhydroxyalkanoate, polyhydroxybutyrate, poly(glycerol sebacate), polyglycolide, polylactide, polycaprolactone, or polyanhydride, or copolymers or derivatives including these and/or other polymers. Alternatively or in addition, the conduit can include polyvinyl chloride. The conduit can be, e.g., weldable transfer tubing.

In some embodiments, the internal diameter of the conduit in the circuit system can be about 5 mm to about 50 mm (e.g., or any of the subranges thereof described herein). In some embodiments, the conduit in the circuit system has a total length of about 1.0 cm to about 75 cm (or any of the subranges of this range described herein). Additional examples of conduits and properties of conduits that can be used in the second circuit system are well-known by those in the art.

In some examples, the circuit system includes one, two, three, four, or five ports. In some examples, the at least one port in the circuit system can be a valve. In other examples, the at least one port in the circuit system can be an injection port or can have a ribbed threading. The at least one port in the circuit system can be any type of port commonly known in the art. In some examples, the circuit system can further include a pump (e.g., a low-turbulence pump, e.g., a peristaltic pump) disposed in the conduit. In some embodiments, the circuit system does not include a holding tank.

In some examples, step (b) includes continuously flowing (e.g., unidirectionally or bidirectionally flowing) a fluid including a recombinant therapeutic protein into the circuit system at a rate of between about 0.05 mL/minute to about 25 mL/minute (or any of the subranges of this range described herein). In some examples, the filtrate collected from the TFVF unit in step (b) includes a concentration of recombinant therapeutic protein of about 0.1 mg/mL to about 100 mg/mL (or any of the subranges of this range described herein). In some examples of these methods, the period of time can be about 5 minutes to about 12 hours (or any of the subranges of this range described herein).

In some examples of these methods, the period of time can be, e.g., about 12 hours to about 72 hours (or any of the subranges of this range described herein).

In some embodiments, the fluid that is continuously flowed into the circuit system including the TFVF unit through one of the at least one port can include a total virus titer of about 10^{-1} to about 10^{-20} (or any of the subranges of this range described herein).

5 In some embodiments, the step of continuously flowing the fluid through the circuit system including a TFVF unit can be performed such that the flux across the filter or one or more filters in the TFVF unit is, e.g., about 10 LMH to about 50 LMH (or any of the subranges of this range described herein).

10 In some embodiments, the step of continuously flowing the fluid through the circuit system including a TFVF unit can be performed such that the pressure across the filter or the one or more filters in the TFVF unit, or the pressure in the system including the TFVF is, e.g., about 2 psi to about 40 psi (or any of the subranges of this range described herein).

15 In some embodiments, the method can further include performing one or more unit operations on a fluid including the recombinant protein. Non-limiting examples of these unit operations include: capturing a recombinant therapeutic protein, purifying a recombinant therapeutic protein, polishing a recombinant therapeutic protein, viral inactivation, viral filtration, adjusting the ionic concentration and/or pH of a fluid containing the recombinant therapeutic protein, filtration of a fluid
20 containing the recombinant therapeutic protein, precipitation and flocculation, aqueous two-phase extraction, lyophilization, and crystallization. Non-limiting aspects of each of these exemplary unit operations are described herein.

25 ***Integrated and Continuous Processes for Manufacturing a Therapeutic Protein Drug Substance***

Provided herein are integrated and continuous processes for manufacturing a therapeutic protein drug substance. These processes include providing a liquid culture medium containing a recombinant therapeutic protein that is substantially free of cells, where the liquid culture medium is fed into a first multi-column
30 chromatography system (MCCS1); capturing the recombinant therapeutic protein in the liquid culture medium using the MCCS1, where the eluate of the MCCS1 containing the recombinant therapeutic protein is continuously fed into a second multi-column chromatography system (MCCS2); purifying and polishing the

recombinant therapeutic protein using the MCCS2, where the eluate from the MCCS2 is a fluid including the recombinant therapeutic protein; processing the fluid including the recombinant therapeutic protein using any of the methods described herein, where the process is integrated and runs continuously from the liquid culture medium to the collected fluid or filtrate including the recombinant therapeutic protein, where the collected fluid or filtrate is the therapeutic protein drug substance. Examples of systems that can be used to perform these processes are shown in Figures 1 and 2 (where asterisks indicate where one or both of a circuit system including tangential flow filtration unit and a tangential flow virus filtration unit can optionally be introduced into the system). For example, one or both of a circuit system including tangential flow filtration unit and a tangential flow viral filtration unit can be used before the performance of a first unit operation, between the performance of a first and second unit operation, between the performance of a second and a third unit operation, between the performance of a third and a fourth unit operation, between the performance of a fourth and a fifth unit operation, between the performance of a fifth and a sixth unit operation, between the performance of a sixth and a seventh unit operation, between the performance of a seventh and an eighth unit operation, and/or between an eighth and a ninth unit operation.

The processes described herein provide continuous and time-efficient production of a therapeutic drug substance from a liquid culture medium. For example, the elapsed time between feeding a fluid (e.g., a liquid culture medium) containing a therapeutic protein into the first MCCS and eluting a therapeutic protein drug substance (containing the therapeutic protein) from the second MCCS can be, e.g., between about 4 hours and about 48 hours, inclusive, e.g., between about 4 hours and about 40 hours, between about 4 hours and about 35 hours, between about 4 hours and about 30 hours, between about 4 hours and about 28 hours, between about 4 hours and about 26 hours, between about 4 hours and about 24 hours, between about 4 hours and about 22 hours, between about 4 hours and about 20 hours, between about 4 hours and about 18 hours, between about 4 hours and about 16 hours, between about 4 hours and about 14 hours, between about 4 hours and about 12 hours, between about 6 hours and about 12 hours, between about 8 hours and about 12 hours, between about 6 hours and about 20 hours, between about 6 hours and about 18 hours, between about 6 hours and about 14 hours, between about 8 hours and about 16 hours, between about 8 hours

and about 14 hours, between about 8 hours and about 12 hours, between about 10 hours and 20 hours, between about 10 hours and 18 hours, between about 10 hours and 16 hours, between about 10 hours and 14 hours, between about 12 hours and about 14 hours, between about 10 hours and about 40 hours, between about 10 hours and about 35 hours, between about 10 hours and about 30 hours, between about 10 hours and about 25 hours, between about 15 hours and about 40 hours, between about 15 hours and about 35 hours, between about 15 hours and about 30 hours, between about 20 hours and about 40 hours, between about 20 hours and about 35 hours, or between about 20 hours and about 30 hours, inclusive. In other examples, the elapsed time between feeding the fluid (e.g., a liquid culture medium) containing a therapeutic protein into the MCCS1 and eluting a therapeutic protein drug substance (containing the therapeutic protein) from the MCCS2 is, e.g., greater than about 4 hours and less than about 40 hours, inclusive, e.g., greater than about 4 hours and less than about 39 hours, about 38 hours, about 37 hours, about 36 hours, about 35 hours, about 34 hours, about 33 hours, about 32 hours, about 31 hours, about 30 hours, about 29 hours, about 28 hours, about 27 hours, about 26 hours, about 25 hours, about 24 hours, about 23 hours, about 22 hours, about 21 hours, about 20 hours, about 19 hours, about 18 hours, about 17 hours, about 16 hours, about 15 hours, about 14 hours, about 13 hours, about 12 hours, about 11 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, or about 4.5 hours, inclusive.

Some exemplary processes do not utilize a holding step (e.g., do not use a reservoir (e.g., break tank) in the entire process). Others may use a maximum of 1, 2, 3, 4, or 5 reservoir(s) (e.g., break tank(s)) in the entire process. Any of the processes described herein can utilize a maximum of 1, 2, 3, 4, or 5 reservoir(s) (e.g., break tank(s)) in the entire process, where each break tank only holds a therapeutic protein for a total time period of, e.g., between about 5 minutes and less than about 6 hours, inclusive, e.g., between about 5 minutes and about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, or about 30 minutes, inclusive. Any of the reservoir(s) (e.g., break tank(s)) used in the processes described herein can have a capacity that is, e.g., between 1 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L,

about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL,

5 about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, about 20 mL, or about 10 mL (inclusive); between about 5 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about

10 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL,

15 about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, about 20 mL, or about 10 mL (inclusive); between about 10 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about

20 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, or about

25 20 mL (inclusive); between about 20 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700

30 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, or about 40 mL (inclusive);

between about 40 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, or about 60 mL (inclusive); between about 60 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, or about 80 mL (inclusive); between about 80 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, or about 100 mL (inclusive); between about 100 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, or

about 120 mL (inclusive); between about 120 mL and about 10,000 L, about 9,500 L,
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 about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about
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 20 about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900
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 30 about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800
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 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L,
 about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950
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 5 about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400
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 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L,
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 15 about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about
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 20 about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about
 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L,
 about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750
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 (inclusive); between about 500 mL and about 10,000 L, about 9,500 L, about 9,000 L,
 25 about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about
 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L,
 about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950
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 about 650 mL, about 600 mL, or about 550 mL (inclusive); between about 550 mL
 30 and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L,
 about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about
 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L,
 about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850

mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, or about 600 mL (inclusive); between about 600 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, or about 650 mL (inclusive); between about 650 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, or about 700 mL (inclusive); between about 700 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, or about 750 mL (inclusive); between about 750 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, about 850 mL, or about 800 mL (inclusive); between about 800 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, about 900 mL, or about 850 mL (inclusive); between about 850 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, about 1,000 L, about 950 mL, or about 900 mL (inclusive); between about 900 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about

2,000 L, about 1,500 L, about 1,000 L, or about 950 mL (inclusive); between about 950 mL and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, about 1,500 L, or about 1,000 L (inclusive); between about 1,000 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, about 2,000 L, or about 1,500 L (inclusive); between about 1,500 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, about 3,000 L, about 2,500 L, or about 2,000 L (inclusive); between about 2,000 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, about 3,500 L, or about 3,000 L (inclusive); between about 3,000 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, about 4,000 L, or about 3,500 L (inclusive); between about 3,500 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, about 4,500 L, or about 4,000 L (inclusive); between about 4,000 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, about 5,000 L, or about 4,500 L (inclusive); between about 4,500 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, about 5,500 L, or about 5,000 L (inclusive); between about 5,000 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, about 6,000 L, or about 5,500 L (inclusive); between about 5,500 L and about 10,000 L, about 9,500 L, about 9,000 L,

about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, about 6,500 L, or about 6,000 L (inclusive); between about 6,000 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, about 7,000 L, or about 6,500 L (inclusive); between about 6,500 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, about 7,500 L, or about 7,000 L (inclusive); between about 7,000 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, about 8,000 L, or about 7,500 L (inclusive); between about 7,500 L and about 10,000 L, about 9,500 L, about 9,000 L, about 8,500 L, or about 8,000 L (inclusive); between about 8,000 L and about 10,000 L, about 9,500 L, about 9,000 L, or about 8,500 L (inclusive); between about 8,500 L and about 10,000 L, about 9,500 L, or about 9,000 L (inclusive); between about 9,000 L and about 10,000 L or about 9,500 L (inclusive); or between about 9,500 L and about 10,000 L (inclusive).

Any reservoir(s) (e.g., break tank(s)) used (in any of the processes described herein) to hold fluid before it enters into the first MCCS can have a capacity that is, e.g., between 1 mL and about 100%, inclusive, e.g., between 1 mL and about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, or about 5%, inclusive, of the loading volume of the first column of the first MCCS. Any of the reservoir(s) (e.g., break tanks(s)) used (in any of the processes described herein) to hold fluid before it enters into the second MCCS (and after the fluid leaves the first MCCS) can have a capacity that is, e.g., between 1 mL and about 100%, inclusive, e.g., between 1 mL and about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, or about 5%, inclusive, of the loading volume of the first column of the second MCCS.

Various additional aspects of these processes are described in detail below and can be used in any combination in the processes provided herein without limitation. Exemplary aspects of the provided processes are described below; however, one skilled in the art will appreciate that additional steps (e.g., the performance of additional unit operations) can be added to the processes described herein and other materials can be used to perform any of the steps of the processes described herein.

Liquid Culture Medium

Liquid culture medium that contains a recombinant therapeutic protein that is substantially free of cells can be derived from any source. For example, the liquid

culture medium can be obtained from a recombinant cell culture (e.g., a recombinant bacterial, yeast, or mammalian cell culture). The liquid culture medium can be obtained from a fed-batch cell (e.g., mammalian cell) culture (e.g., a fed-batch bioreactor containing a culture of mammalian cells that secrete the recombinant therapeutic protein) or a perfusion cell (e.g., mammalian cell) culture (e.g., a perfusion bioreactor containing a culture of mammalian cells that secrete the recombinant therapeutic protein). The liquid culture medium can also be a clarified liquid culture medium from a culture of bacterial or yeast cells that secrete the recombinant therapeutic protein.

Liquid culture medium obtained from a recombinant cell culture can be filtered or clarified to obtain a liquid culture medium that is substantially free of cells and/or viruses. Methods for filtering or clarifying a liquid culture medium in order to remove cells are known in the art (e.g., 0.2- μ m filtration and filtration using an Alternating Tangential Flow (ATFTM) system).

Additional exemplary methods for filtering or clarifying a liquid culture medium include, e.g., immobilized cell separation (e.g., hollow fiber membranes, flat plate membranes, gel encapsulation, ceramic matrices, and fluidized beds, e.g., as described in Kührtreiber et al., Springer Science & Business Media, 2013; Meuwly et al., *Biotechnol. Adv.* 25(1):45-56, 2007; Jen et al., *Biotechnol. Bioeng.* 50(4):357-364, 1996; Piret et al., *Biotechnol. Adv.* 8(4):763-783, 1990; and Tyo et al., *Enzyme Microb. Technol.* 9(9):514-520, 1987); density-based cell separation (e.g., gravitational settling (e.g., gravitational settling performed using conical sedimentation columns (e.g., as described in Kitano et al., *Appl. Microbiol. Biotechnol.* 24(4):282-286, 1986; Shintani et al., *Cytotechnology* 6(3):197-208, 1991), intra-reactor settling zone (e.g., as described in Takazawa et al., *Appl. Microbiol. Biotechnol.* 32(3):280-284, 1989), a Dortmund settler (e.g., as described in Hülscher et al., *Biotechnol. Bioeng.* 39(4):442-446, 1992), and inclined settlers (e.g., as described in Boycott et al., *Nature* 104:532, 1920; Acrivos et al., *J. Fluid Mechanics* 92(3):435-457, 1979; Searles et al., *Biotechnol. Prog.* 10(2):198-206, 1994; Kohara et al., *J. Chem. Eng. Japan* 28(6):703-707, 1995; Shen et al., *Biotechnol. Prog.* 27(5):1282-1296, 2011; Wang et al., *Biotechnol. Prog.* 26(5):1361-1366, 2010; Pohlscheidt et al., *Biotechnol. Prog.* 29(1):222-229, 2013; Hecht et al., *Biotechnol. Prog.* 30(3):607-615, 2014)); centrifugation (e.g., continuous centrifugation (e.g., as

described in Hamamoto et al., *J. Ferment. Bioeng.* 67(3):190-194, 1989; Tokashiki et al., *Cytotechnology* 3(3):239-244, 1990; and Takamatsu et al., *Appl. Microbiol. Biotechnol.* 45(4):454-457, 1996), Centritech centrifugation (commercially available from Pneumatic Scale Angelus and as described in, e.g., Johnson et al., *Biotechnol. Prog.* 12(6):855-864, 1996; Kim et al., *Biotechnol. Prog.* 23 (5):1186-1197, 2007; Kim et al., *Biotechnol. Prog.* 24(1):166-174, 2008; Hecht et al., *Biotechnol. Prog.* 30 (3):607-615, 2014; Chotteau et al., *Perfusion Processes*, In: Al-Rubeai M (Ed.), *Animal Cell Culture*, Springer, Cham, Switzerland, 2015), and centrifuges that utilize continuous counterflow (e.g., as described in Pattasseril et al., *BioProcess Int.* 11(3):38-47, 2013)); acoustic settling (e.g., using an acoustic settler, e.g., the acoustic settlers described in Kilburn et al., *Biotechnol Bioeng.* 34(4):559-562, 1989; Shirgaonkar et al., *Biotechnol. Adv.* 22(6):433-444, 2004; Doblhoff-Dier et al., *Biotechnol. Prog.* 10(4):428-432, 1994; Gaida et al., *Biotechnol. Prog.* 12(1):73-76, 1996; Bierau et al., *J. Biotechnol.* 62 (3):195-207, 1998; Crowley et al., *BioProcess Int.* 2(3):46-50, 2004; Gorenflo et al., *Biotechnol. Prog.* 19(1):30-36, 2003; Pui et al., *Biotechnol. Prog.* 11(2):146-152, 1995; Gorenflo et al., *Biotechnol. Bioeng.* 80 (4):438-444, 2002; Dalm et al., *Biotechnol. Bioeng.* 88(5):547-557, 2004; Gorenflo et al., *Biotechnol. Prog.* 19(1):30-36, 2005); and hydrocyclone separation (e.g., using the hydroclone separators described in Elsayed et al., *Int. J. Biotechnol. Wellness Indust.* 2(4):153, 2013; Castilho et al., *Continuous animal cell perfusion processes: the first step toward integrated continuous biomanufacturing*, in Subramanian G. (Ed.), *Continuous Processing in Pharmaceutical Manufacturing*, Wiley-VCH, Weinheim, Germany, 2014; Jockwer et al., *The use of hydroclones for mammalian cell retention in perfusion bioreactors*, in *Animal Cell Technology: From Target to Market*, Springer, pp. 301-306, 2001; Pinto et al., *Cytotechnology* 56(1):57-67, 2008; Elsayed et al., *Eng. Life Sci.* 6(4):347-354, 2006; Elsayed et al., *BMC Proceedings* 5(Supp. 8):p65, 2001; Castilho et al., *Animal cell separation*, in Castilho et al. (Ed.), *Animal Cell Technology: From Biopharmaceuticals to Gene Therapy*, Taylor & Francis, New York, NY, pp. 273-294, 2008).

Additional exemplary methods for filtering or clarifying a liquid culture medium include, e.g., size-based cell separation, e.g., performed using spin filtration (e.g., spin filtration as described in Himmelfarb et al., *Science* 164(3879):555-557, 1969; Tolbert et al., *In Vitro* 17(10):885-890, 1981; Reuveny et al., *J. Immunol.*

Methods 86(1):61-69, 1986; Avgerinos et al., *Nat. Biotechnol.* 8 (1):54-58, 1990; Kim et al., *Biotechnol. Prog.* 24(1):166-174, 2008; Bierau et al., *J. Biotechnol.* 62 (3):195-207, 1998; Castilho et al., *Biotechnol. Prog.* 18(4):776-781, 2002; Deo et al., *Biotechnol. Prog.* 12(1):57-64, 1996; Emery et al., *Appl. Microbiol. Biotechnol.* 43(6):1028-1033, 1995; Hecht et al., *Biotechnol. Prog.* 30(3):607-615., 2014; Vallez-Chetreaunu et al., *J. Biotechnol.* 130(3):265-273, 2007; Yabannavar et al., *Biotechnol. Bioeng.* 40 (8):925-933, 1992; Yabannavar et al., *Biotechnol. Bioeng.* 40 (8):925-933, 1994; Deo et al., *Biotechnol. Prog.* 12 (1):57-64 1996; and Castilho et al., *Biotechnol. Prog.* 18(4):776-781, 2002); hollow-fiber filtration (e.g., placement of hollow-fiber cartridges placed directly within a bioreactor (e.g., as described in Seamans et al., *J. Ferment. Bioeng.* 70(4):241-245, 1990, and Kyung et al., *Cytotechnology* 14(3):183-190, 1994), tangential-flow-filtration (TFF) (e.g., as described in Brennan et al., *Biotechnol. Tech.* 1(3):169-174, 1987; Velez et al., *Biotechnol. Bioeng.* 33(7):938-940, 1989; de la Broise et al., *Biotechnol. Bioeng.* 38(7):781-787, 1991; Greenfield et al., *Bioprocess Eng.* 6(5):213-219, 1991; Kawahara et al., *Cytotechnology* 14(1):61-66, 1994; Karst et al., *Biochem. Eng. J.* 110:17-26, 2016; Martin et al., *BMC Proceedings* 9:p1, 2015; Clincke et al., *Biotechnol. Prog.* 29 (3):754-767, 2013); alternating tangential flow (ATF) filtration (e.g., as described in Kelly et al., *Biotechnol. Prog.* 30 (6):1291-1300, 2014; Karst et al., *Biochem. Eng. J.* 110:17-26, 2016; Kim et al., *Cytotechnology* pp. 1-10, 2015; Xu et al., *J. Biotechnol.* 231:149-159, 2016; Warikoo et al., *Biotechnol. Bioeng.* 109(12):3018-3029, 2012; Clincke et al., *Biotechnol. Prog.* 29(3):754-767, 2013; Wright et al., *Bioprocess International*, Volume 13, March 10, 2015; Yang et al., *Biotechnol. Prog.* 30(3):616-625, 2014; and Padawer et al., *Biotechnol. Prog.* 29(3):829-832, 2013); use of a disposable floating filter (e.g., Tao et al., *Biotechnol. Prog.* 27(3):824-829, 2011); vortex flow filtration (VFF) (e.g., as described in Mercille et al., *Biotechnol. Bioeng.* 43 (9):833-846, 1994; Mercille et al., *Biotechnol. Bioeng.* 67 (4):435-450, 2000; and Roth et al., *Pharmaceutical Technology* 21(10), 1997); and continuous depth filtration (as described in Vogel et al., *Biotechnol. Bioeng.* 109 (12):3049-3058, 2012, and Klutz et al., *Chemical Engineering Processing: Process Intensification* 102:88-101, 2015).

Recombinant cells can also be removed from liquid culture medium using centrifugation and removing the supernatant that is liquid culture medium that is substantially free of cells, or by allowing the cells to settle to the gravitational bottom

of a container (e.g., bioreactor) containing the liquid culture medium, and removing the liquid culture medium (the liquid culture medium that is substantially free of cells) that is distant from the settled recombinant cells.

5 The liquid culture medium can be obtained from a culture of recombinant cells (e.g., recombinant bacteria, yeast, or mammalian cells) producing any of the recombinant therapeutic proteins described herein. Some examples of any of the processes described herein can further include a step of culturing recombinant cells (e.g., recombinant bacteria, yeast, or mammalian cells) that produce the recombinant therapeutic protein.

10 The liquid culture medium can be any of the types of liquid culture medium described herein or known in the art. For example, the liquid culture medium can be selected from the group of: animal-derived component free liquid culture medium, serum-free liquid culture medium, serum-containing liquid culture medium, chemically-defined liquid culture medium, and protein-free liquid culture medium. In
15 any of the processes described herein, a liquid culture medium obtained from a culture can be diluted by addition of a second fluid (e.g., a buffer) before it is fed into the first MCCS (e.g., first PCCS).

The liquid culture medium containing a recombinant therapeutic protein that is substantially free of cells can be stored (e.g., at a temperature below about 15 °C (e.g.,
20 below about 10 °C, below about 4 °C, below about 0 °C, below about -20 °C, below about -50 °C, below about -70 °C, or below about -80 °C) for at least 1 day (e.g., at least about 2 days, at least about 5 days, at least about 10 days, at least about 15 days, at least about 20 days, or at least about 30 days) prior to feeding the liquid culture medium into the first MCCS (e.g., first PCCS). Alternatively, in some examples the
25 liquid culture medium is fed into the first MCCS (e.g., first PCCS) directly from a bioreactor (e.g., fed into the first MCCS (e.g., first PCCS) directly from the bioreactor after a filtering or clarification step).

Recombinant Therapeutic Proteins

Non-limiting examples of recombinant therapeutic proteins that can be
30 produced by the methods provided herein include immunoglobulins (including light and heavy chain immunoglobulins, antibodies, or antibody fragments (e.g., any of the antibody fragments described herein), enzymes (e.g., a galactosidase (e.g., an alpha-galactosidase), Myozyme, or Cerezyme), proteins (e.g., human erythropoietin, tumor

necrosis factor (TNF), or an interferon alpha or beta), or immunogenic or antigenic proteins or protein fragments (e.g., proteins for use in a vaccine). The recombinant therapeutic protein can be an engineered antigen-binding polypeptide that contains at least one multifunctional recombinant protein scaffold (see, e.g., the recombinant antigen-binding proteins described in Gebauer et al., *Current Opin. Chem. Biol.* 13:245-255, 2009; and U.S. Patent Application Publication No. 2012/0164066 (herein incorporated by reference in its entirety)). Non-limiting examples of recombinant therapeutic proteins that are antibodies include: panitumumab, omalizumab, abagovomab, abciximab, actoxumab, adalimumab, adecatumumab, afelimomab, afutuzumab, alacizumab, alacizumab, alemtuzumab, alirocumab, altumomab, amatuximab, amatuximab, anatumomab, anrukinzumab, apolizumab, arcitumomab, atinumab, tocilizumab, basilizimab, bectumomab, belimumab, bevacizumab, besilesomab, bezlotoxumab, biciromab, canakinumab, certolizumab, cetuximab, cixutumumab, daclizumab, denosumab, densumab, eculizumab, edrecolomab, efalizumab, efungumab, epratuzumab, ertumaxomab, etaracizumab, figitumumab, golimumab, ibritumomab tiuxetan, igovomab, imgatuzumab, infliximab, inolimomab, inotuzumab, labetuzumab, lebrikizumab, moxetumomab, natalizumab, obinutuzumab, oregovomab, palivizumab, panitumumab, pertuzumab, ranibizumab, rituximab, tocilizumab, tositumomab, tralokinumab, tucotuzumab, trastuzumab, veltuzumab, zalutumumab, and zatuximab. Additional examples of recombinant therapeutic antibodies that can be produced by the methods described herein are known in the art. Additional non-limiting examples of recombinant therapeutic proteins that can be produced by the present methods include: alglucosidase alfa, laronidase, abatacept, galsulfase, lutropin alfa, antihemophilic factor, agalsidase beta, interferon beta-1a, darbepoetin alfa, tenecteplase, etanercept, coagulation factor IX, follicle stimulating hormone, interferon beta-1a, imiglucerase, dornase alfa, epoetin alfa, insulin or insulin analogs, mecasermin, factor VIII, factor VIIa, anti-thrombin III, protein C, human albumin, erythropoietin, granulocyte colony stimulating factor, granulocyte macrophage colony stimulating factor, interleukin-11, laronidase, idursuphase, galsulphase, α -1-proteinase inhibitor, lactase, adenosine deaminase, tissue plasminogen activator, thyrotropin alpha (e.g., Thyrogen®) and alteplase. Additional examples of recombinant proteins that can be produced by the present methods include acid α -glucosidase, alglucosidase alpha (e.g., Myozyme® and Lumizyme®),

α -L-iduronidase (e.g., Aldurazyme®), iduronate sulfatase, heparan N-sulfatase, galactose-6-sulfatase, acid β -galactosidase, β -glucuronidase, N-acetylglucosamine-1-phosphotransferase, α -N-acetylgalactosaminidase, acid lipase, lysosomal acid ceramidase, acid sphingomyelinase, β -glucosidase (e.g., Cerezyme® and Ceredase®),
 5 galactosylceramidase, α -galactosidase-A (e.g., Fabrazyme®), acid β -galactosidase, β -galactosidase, neuraminidase, hexosaminidase A, and hexosaminidase B.

A secreted, soluble recombinant therapeutic protein can be recovered from the liquid culture medium (e.g., a first and/or second liquid culture medium) by removing or otherwise physically separating the liquid culture medium from the cells (e.g.,
 10 mammalian cells). A variety of different methods for removing liquid culture medium from cells (e.g., mammalian cells) are known in the art, including, for example, centrifugation, filtration, pipetting, and/or aspiration. The secreted recombinant therapeutic protein can then be recovered and further purified from the liquid culture medium using a variety of biochemical techniques including various
 15 types of chromatography (e.g., affinity chromatography, molecular sieve chromatography, cation exchange chromatography, or anion exchange chromatography) and/or filtration (e.g., molecular weight cut-off filtration).

Multi-Column Chromatography Systems

20 The processes described herein include the use of two or more (e.g., two, three, four, five, or six) multi-column chromatography systems (MCCSs). A MCCS can include two or more chromatography columns, two or more chromatographic membranes, or a combination of at least one chromatography column and at least one chromatographic membrane. In non-limiting examples, a MCCS (e.g., the first and/or
 25 second MCCS described in any of the processes herein) can include four chromatographic columns, three chromatographic columns and a chromatographic membrane, three chromatographic columns, two chromatographic columns, two chromatographic membranes, and two chromatographic columns and one chromatographic membrane. Additional examples of combinations of
 30 chromatography columns and/or chromatographic membranes can be envisioned for use in an MCCS (e.g., a first and/or second MCCS) by one skilled in the art without limitation. The individual chromatography columns and/or chromatographic membranes present in a MCCS can be identical (e.g., have the same shape, volume,

resin, capture mechanism, and unit operation), or can be different (e.g., have one or more of a different shape, volume, resin, capture mechanism, and unit operation). The individual chromatography column(s) and/or chromatographic membrane(s) present in a MCCS (e.g., the first and/or second MCCS) can perform the same unit operation (e.g., the unit operation of capturing, purifying, or polishing) or different unit operations (e.g., different unit operations selected from, e.g., the group of capturing, purifying, polishing, inactivating viruses, adjusting the ionic concentration and/or pH of a fluid containing the recombinant therapeutic protein, and filtering).

The one or more chromatography column(s) that can be present in an MCCS (e.g., present in the first and/or second MCCS) can have a resin volume of, e.g., at least about 2 mL, at least about 5 mL, at least about 10 mL, at least about 15 mL, at least about 20 mL, at least about 25 mL, at least about 30 mL, at least about 35 mL, at least about 40 mL, at least about 45 mL, at least about 50 mL, at least about 55 mL, at least about 60 mL, at least about 65 mL, at least about 70 mL, at least about 75 mL, at least about 80 mL, at least about 85 mL, at least about 90 mL, at least about 95 mL, at least about 100 mL, at least about 110 mL, at least about 120 mL, at least about 130 mL, at least about 140 mL, at least about 150 mL, at least about 160 mL, at least about 170 mL, at least about 180 mL, at least about 190 mL, at least about 200 mL, at least about 250 mL, at least about 300 mL, at least about 350 mL, at least about 400 mL, at least about 450 mL, at least about 500 mL, at least about 550 mL, at least about 600 mL, at least about 650 mL, at least about 700 mL, at least about 750 mL, at least about 800 mL, at least about 850 mL, at least about 900 mL, at least about 950 mL, at least about 1 L, at least about 10 L, at least about 20 L, at least about 30 L, at least about 40 L, at least about 50 L, at least about 60 L, at least about 70 L, at least about 80 L, at least about 90 L, at least about 100 L, at least about 110 L, at least about 120 L, at least about 130 L, at least about 140 L, at least about 150 L, at least about 160 L, at least about 170 L, at least about 180 L, at least about 190 L, or at least about 200 L.

The one or more chromatography column(s) that can be present in an MCCS (e.g., present in the first and/or second MCCS) can have a resin volume of between about 2 mL and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL, about 500

mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL, about 90 mL, about
 80 mL, about 70 mL, about 60 mL, about 50 mL, about 45 mL, about 40 mL, about
 35 mL, about 30 mL, about 25 mL, about 20 mL, about 15 mL, about 10 mL, or about
 5 mL; between about 5 mL and about 200 L, about 180 L, about 160 L, about 140 L,
 5 about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L,
 about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL,
 about 500 mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL, about 90
 mL, about 80 mL, about 70 mL, about 60 mL, about 50 mL, about 45 mL, about 40
 mL, about 35 mL, about 30 mL, about 25 mL, about 20 mL, about 15 mL, or about 10
 10 mL; between about 10 mL and about 200 L, about 180 L, about 160 L, about 140 L,
 about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L,
 about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL,
 about 500 mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL, about 90
 mL, about 80 mL, about 70 mL, about 60 mL, about 50 mL, about 45 mL, about 40
 15 mL, about 35 mL, about 30 mL, about 25 mL, about 20 mL, or about 15 mL; between
 about 15 mL and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L,
 about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L,
 about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL, about 500
 mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL, about 90 mL, about
 20 80 mL, about 70 mL, about 60 mL, about 50 mL, about 45 mL, about 40 mL, about
 35 mL, about 30 mL, about 25 mL, or about 20 mL; between about 20 mL and about
 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L,
 about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL,
 about 800 mL, about 700 mL, about 600 mL, about 500 mL, about 400 mL, about 300
 25 mL, about 200 mL, about 100 mL, about 90 mL, about 80 mL, about 70 mL, about 60
 mL, about 50 mL, about 45 mL, about 40 mL, about 35 mL, about 30 mL, or about 25
 mL; between about 25 mL and about 200 L, about 180 L, about 160 L, about 140 L,
 about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L,
 about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL,
 30 about 500 mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL, about 90
 mL, about 80 mL, about 70 mL, about 60 mL, about 50 mL, about 45 mL, about 40
 mL, about 35 mL, or about 30 mL; between about 30 mL and about 200 L, about 180
 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about

40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL,
 about 700 mL, about 600 mL, about 500 mL, about 400 mL, about 300 mL, about 200
 mL, about 100 mL, about 90 mL, about 80 mL, about 70 mL, about 60 mL, about 50
 mL, about 45 mL, about 40 mL, or about 35 mL; between about 35 mL and about 200
 5 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L,
 about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL,
 about 800 mL, about 700 mL, about 600 mL, about 500 mL, about 400 mL, about 300
 mL, about 200 mL, about 100 mL, about 90 mL, about 80 mL, about 70 mL, about 60
 mL, about 50 mL, about 45 mL, or about 40 mL; between about 40 mL and about 200
 10 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L,
 about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL,
 about 800 mL, about 700 mL, about 600 mL, about 500 mL, about 400 mL, about 300
 mL, about 200 mL, about 100 mL, about 90 mL, about 80 mL, about 70 mL, about 60
 mL, about 50 mL, or about 45 mL; between about 45 mL and about 200 L, about 180
 15 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about
 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL,
 about 700 mL, about 600 mL, about 500 mL, about 400 mL, about 300 mL, about 200
 mL, about 100 mL, about 90 mL, about 80 mL, about 70 mL, about 60 mL, or about
 50 mL; between about 50 mL and about 200 L, about 180 L, about 160 L, about 140
 20 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10
 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL,
 about 500 mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL, about 90
 mL, about 80 mL, about 70 mL, or about 60 mL; between about 60 mL and about 200
 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L,
 25 about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL,
 about 800 mL, about 700 mL, about 600 mL, about 500 mL, about 400 mL, about 300
 mL, about 200 mL, about 100 mL, about 90 mL, about 80 mL, or about 70 mL;
 between about 70 mL and about 200 L, about 180 L, about 160 L, about 140 L, about
 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about
 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL, about 500
 30 mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL, about 90 mL, or
 about 80 mL; between about 80 mL and about 200 L, about 180 L, about 160 L, about
 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L,

about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about
 600 mL, about 500 mL, about 400 mL, about 300 mL, about 200 mL, about 100 mL,
 or about 90 mL; between about 90 mL and about 200 L, about 180 L, about 160 L,
 about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20
 5 L, about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL,
 about 600 mL, about 500 mL, about 400 mL, about 300 mL, about 200 mL, or about
 100 mL; between about 100 mL and about 200 L, about 180 L, about 160 L, about
 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L,
 about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about 700 mL, about
 10 600 mL, about 500 mL, about 400 mL, about 300 mL, or about 200 mL; between
 about 200 mL and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L,
 about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L,
 about 1 L, about 900 mL, about 800 mL, about 700 mL, about 600 mL, about 500
 mL, about 400 mL, or about 300 mL; about 300 mL and about 200 L, about 180 L,
 15 about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40
 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about
 700 mL, about 600 mL, about 500 mL, or about 400 mL; between about 400 mL and
 about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about
 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900
 20 mL, about 800 mL, about 700 mL, about 600 mL, or about 500 mL; between about
 500 mL and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1
 L, about 900 mL, about 800 mL, about 700 mL, or about 600 mL; between about 600
 mL and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100
 25 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L,
 about 900 mL, about 800 mL, or about 700 mL; between about 700 mL and about 200
 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L,
 about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL,
 or about 800 mL; between about 800 mL and about 200 L, about 180 L, about 160 L,
 30 about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20
 L, about 10 L, about 5 L, about 1 L, or about 900 mL; between about 900 mL and
 about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about
 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, or about 1 L;

between about 1 L and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, or about 5 L; between about 5 L and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, or about 10 L; between 10 L and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, or about 20 L; between 20 L and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, or about 40 L; between about 40 L and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, or about 60 L; between about 60 L and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, or about 80 L; between about 80 L and about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, or about 100 L; between about 100 L and about 200 L, about 180 L, about 160 L, about 140 L, or about 120 L; between about 120 L and about 200 L, about 180 L, about 160 L, or about 140 L; between about 140 L and about 200 L, about 180 L, or about 160 L; between about 160 L and about 200 L or about 180 L; or about 180 L to about 200 L.

The one or more chromatography column(s) in an MCCS (e.g., the first and/or second MCCS) used in any of the processes described herein can have the substantially the same resin volume or can have different resin volumes. The flow rate used for the one or more chromatography column(s) in an MCCS (e.g., the first and/or second MCCS) can be, e.g., between about 0.2 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about 80 mL/minute, about 70 mL/minute, about 60 mL/minute, about 50 mL/minute, about 40 mL/minute, about 30 mL/minute, about 25 mL/minute, about 20 mL/minute, about 15 mL/minute, about 10 mL/minute, about 5 mL/minute, about 2 mL/minute, or about 1 mL/minute; between about 1 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, about 700

mL/minute, about 600 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 about 90 mL/minute, about 80 mL/minute, about 70 mL/minute, about 60 mL/minute,
 5 about 50 mL/minute, about 40 mL/minute, about 30 mL/minute, about 25 mL/minute,
 about 20 mL/minute, about 15 mL/minute, about 10 mL/minute, about 5 mL/minute,
 or about 2 mL/minute; between about 2 mL/minute to about 100 L/minute, about 80
 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800
 10 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute,
 about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300
 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute,
 about 100 mL/minute, about 90 mL/minute, about 80 mL/minute, about 70
 mL/minute, about 60 mL/minute, about 50 mL/minute, about 40 mL/minute, about 30
 15 mL/minute, about 25 mL/minute, about 20 mL/minute, about 15 mL/minute, about 10
 mL/minute, or about 5 mL/minute; between about 5 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900
 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute,
 20 about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350
 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute,
 about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about 80
 mL/minute, about 70 mL/minute, about 60 mL/minute, about 50 mL/minute, about 40
 mL/minute, about 30 mL/minute, about 25 mL/minute, about 20 mL/minute, about 15
 25 mL/minute, or about 10 mL/minute; between about 10 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900
 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute,
 about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350
 30 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute,
 about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about 80
 mL/minute, about 70 mL/minute, about 60 mL/minute, about 50 mL/minute, about 40
 mL/minute, about 30 mL/minute, about 25 mL/minute, about 20 mL/minute, or about

15 mL/minute; between about 15 mL/minute to about 100 L/minute, about 80
 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800
 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute,
 5 about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300
 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute,
 about 100 mL/minute, about 90 mL/minute, about 80 mL/minute, about 70
 mL/minute, about 60 mL/minute, about 50 mL/minute, about 40 mL/minute, about 30
 mL/minute, about 25 mL/minute, or about 20 mL/minute; between about 20
 10 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
 15 mL/minute, about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about
 80 mL/minute, about 70 mL/minute, about 60 mL/minute, about 50 mL/minute, about
 40 mL/minute, about 30 mL/minute, or about 25 mL/minute; between about 25
 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 20 about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
 mL/minute, about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about
 80 mL/minute, about 70 mL/minute, about 60 mL/minute, about 50 mL/minute, about
 25 40 mL/minute, or about 30 mL/minute; between about 30 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900
 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute,
 about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350
 30 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute,
 about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about 80
 mL/minute, about 70 mL/minute, about 60 mL/minute, about 50 mL/minute, or about
 40 mL/minute; between about 40 mL/minute to about 100 L/minute, about 80

L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800
 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute,
 about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300
 5 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute,
 about 100 mL/minute, about 90 mL/minute, about 80 mL/minute, about 70
 mL/minute, about 60 mL/minute, or about 50 mL/minute; between about 50
 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 10 about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
 mL/minute, about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about
 80 mL/minute, about 70 mL/minute, or about 60 mL/minute; between about 60
 15 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200
 20 mL/minute, about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, about
 80 mL/minute, or about 70 mL/minute; between about 70 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900
 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute,
 25 about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350
 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute,
 about 150 mL/minute, about 100 mL/minute, about 90 mL/minute, or about 80
 mL/minute; between about 80 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 30 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, about 700
 mL/minute, about 600 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, or

about 90 mL/minute; between about 90 mL/minute to about 100 L/minute, about 80
 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800
 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute,
 5 about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300
 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, or
 about 100 mL/minute; between about 100 mL/minute to about 100 L/minute, about 80
 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800
 10 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute,
 about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300
 mL/minute, about 250 mL/minute, about 200 mL/minute, or about 150 mL/minute;
 between about 150 mL/minute to about 100 L/minute, about 80 L/minute, about 60
 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 15 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, about 700
 mL/minute, about 600 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, or about 200 mL/minute; between about 200 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900
 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute,
 about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350
 mL/minute, about 300 mL/minute, or about 250 mL/minute; between about 250
 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 25 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600
 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute,
 about 350 mL/minute, or about 300 mL/minute; between about 300 mL/minute to
 about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about
 30 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900
 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute,
 about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, or about 350
 mL/minute; between about 350 mL/minute to about 100 L/minute, about 80 L/minute,

about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute, about 450 mL/minute, or about 400 mL/minute; between about 400 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute, about 500 mL/minute, or about 450 mL/minute; between about 450 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, about 700 mL/minute, about 600 mL/minute, or about 500 mL/minute; between about 500 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 900 mL/minute, about 800 mL/minute, or about 700 mL/minute; between about 700 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, or about 900 mL/minute; between about 900 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, or about 1 L/minute; between about 1 L/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, or about 5 L/minute; about 5 L/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, or about 10 L/minute; between about 10 L/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, or about 20 L/minute; between about 20 L/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, or about 40 L/minute; between about 40 L/minute to about 100 L/minute, about 80 L/minute, or

about 60 L/minute; between about 60 L/minute to about 100 L/minute or about 80 L/minute; or about 80 L/minute to about 100 L/minute.

The one or more chromatography column (s) in an MCCS (e.g., the first and/or second MCCS) can have substantially the same shape or can have substantially different shapes. For example, the one or more chromatography column(s) in an MCCS (e.g., in the first and/or second MCCS) can have substantially the shape of a circular cylinder or can have substantially the same shape of an oval cylinder.

The one or more chromatographic membrane(s) that can be present in an MCCS (e.g., present in the first and/or second MCCS) can have a bed volume of, e.g., between about 1 mL and about 10 L, about 9.5 L, about 9 L, about 8.5 L, about 8 L, about 7.5 L, about 7 L, about 6.5 L, about 6 L, about 5.5 L, about 5 L, about 4.5 L, about 4 L, about 3.5 L, about 3 L, about 2.5 L, about 2 L, about 1.5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 475 mL, about 450 mL, about 425 mL, about 400 mL, about 375 mL, about 350 mL, about 325 mL, about 300 mL, about 275 mL, about 250 mL, about 225 mL, about 200 mL, about 175 mL, about 150 mL, about 125 mL, about 100 mL, about 95 mL, about 90 mL, about 85 mL, about 80 mL, about 75 mL, about 70 mL, about 65 mL, about 60 mL, about 55 mL, about 50 mL, about 45 mL, about 40 mL, about 35 mL, about 30 mL, about 25 mL, about 20 mL, about 15 mL, about 10 mL, or about 5 mL (inclusive); between about 2 mL and about 10 L, about 9.5 L, about 9 L, about 8.5 L, about 8 L, about 7.5 L, about 7 L, about 6.5 L, about 6 L, about 5.5 L, about 5 L, about 4.5 L, about 4 L, about 3.5 L, about 3 L, about 2.5 L, about 2 L, about 1.5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 475 mL, about 450 mL, about 425 mL, about 400 mL, about 375 mL, about 350 mL, about 325 mL, about 300 mL, about 275 mL, about 250 mL, about 225 mL, about 200 mL, about 175 mL, about 150 mL, about 125 mL, about 100 mL, about 95 mL, about 90 mL, about 85 mL, about 80 mL, about 75 mL, about 70 mL, about 65 mL, about 60 mL, about 55 mL, about 50 mL, about 45 mL, about 40 mL, about 35 mL, about 30 mL, about 25 mL, about 20 mL, about 15 mL, about 10 mL, or about 5 mL (inclusive); between about 5 mL and about 10 L, about 9.5 L, about 9 L, about 8.5 L, about 8 L, about 7.5 L, about 7 L, about 6.5 L, about 6 L, about 5.5 L, about 5 L,

about 4.5 L, about 4 L, about 3.5 L, about 3 L, about 2.5 L, about 2 L, about 1.5 L,
 about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750
 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL,
 about 475 mL, about 450 mL, about 425 mL, about 400 mL, about 375 mL, about 350
 5 mL, about 325 mL, about 300 mL, about 275 mL, about 250 mL, about 225 mL,
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 about 7.5 L, about 7 L, about 6.5 L, about 6 L, about 5.5 L, about 5 L, about 4.5 L,
 30 about 4 L, about 3.5 L, about 3 L, about 2.5 L, about 2 L, about 1.5 L, about 1 L,
 about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, or about
 700 mL (inclusive); between about 700 mL and about 10 L, about 9.5 L, about 9 L,
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about 5 L, about 4.5 L, about 4 L, about 3.5 L, about 3 L, about 2.5 L, about 2 L,
 about 1.5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL,
 or about 750 mL (inclusive); between about 750 mL and about 10 L, about 9.5 L,
 about 9 L, about 8.5 L, about 8 L, about 7.5 L, about 7 L, about 6.5 L, about 6 L,
 5 about 5.5 L, about 5 L, about 4.5 L, about 4 L, about 3.5 L, about 3 L, about 2.5 L,
 about 2 L, about 1.5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, or
 about 800 mL (inclusive); between about 800 mL and about 10 L, about 9.5 L, about
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 10 about 1.5 L, about 1 L, about 950 mL, about 900 mL, or about 850 mL (inclusive);
 between about 850 mL and about 10 L, about 9.5 L, about 9 L, about 8.5 L, about 8 L,
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One or more (e.g., three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, or twenty-four) different types of buffer can be employed during the use of the two or more MCCSs in any of the processes described herein.

As is known in the art, the one or more types of buffer used in the two or more MCCSs used in the processes described herein will depend on the resin present in the chromatography column(s) and/or the chromatographic membrane(s) of the two or more MCCSs (e.g., the first and second MCCSs), the recombinant therapeutic protein, and unit operation (e.g., any of the exemplary unit operations described herein) performed by the specific chromatography column(s) and/or chromatography membranes of the two or more MCCSs. The volume and type of buffer employed during the use of the two or more MCCSs in any of the processes described herein can also be determined by one skilled in the art (e.g., discussed in more detail below). For

example, the volume and type(s) of buffer employed during the use of the two or more MCCSs in any of the processes described herein can be chosen in order to optimize one or more of the following in the recombinant protein drug product: the overall yield of recombinant therapeutic protein, the activity of the recombinant therapeutic protein, the level of purity of the recombinant therapeutic protein, and the removal of biological contaminants from a fluid containing the recombinant therapeutic protein (e.g., absence of active viruses, mycobacteria, yeast, bacteria, or mammalian cells).

The first and/or second MCCS can be a periodic counter current chromatography system (PCCS). A PCCS can, e.g., include two or more chromatography columns (e.g., three columns or four columns) that are switched in order to allow for the continuous elution of recombinant therapeutic protein from the two or more chromatography columns. A PCCS can include two or more chromatography columns, two or more chromatographic membranes, or at least one chromatographic column and at least one chromatographic membrane. A column operation generally consists of the load, wash, eluate, and regeneration steps. In PCCSs, multiple columns are used to run the same steps discretely and continuously in a cyclic fashion. Since the columns are operated in series, the flow through and wash from one column is captured by another column. This unique feature of PCCSs allows for loading of the resin close to its static binding capacity instead of to the dynamic binding capacity, as is typical during batch mode chromatography. An example of the three column-switching technique used in a PCCS containing three columns is shown in Figure 3. A cycle is defined as three complete column operations resulting in an elution pool from each of the three columns used in the column-switching technique. Once all the steps in the cycle are completed, the cycle is re-started. As a result of the continuous cycling and elution, fluid entering a PCCS is processed continuously, and the eluate containing recombinant therapeutic protein is continuously produced.

To advance from one step to another in a PCCS cycle, such as the exemplary cycle shown in Figure 3, a column-switching strategy is employed. The column switching method employs two automated switching operations per column in the three-columns in the exemplary PCCS system shown in Figure 3, the first of which is related to the initial product breakthrough, while the second coincides with column

saturation. The determination of when the column switching operations should take place is determined by monitoring the recombinant therapeutic protein concentration (e.g., monitoring performed by UV monitoring) in the eluate from each chromatography column present in a PCCS. For example, column switching can be determined by any PAT tool capable of in-line measurement of product concentration with feedback control. The PAT tool is capable of real-time in-line measurement of product concentration with feedback control.

Figure 4 depicts an example of column switching in an exemplary PCCS based on the UV absorbance difference (ΔUV) between the feed inlet and column outlet. For example, during column loading (Step 1; Figure 3), the PCC control system determines the impurity baseline level when the absorbance stabilizes. As the product breaks through (Step 2; Figure 3), there is an increase in the outlet UV signal above the impurity baseline. At the point when ΔUV has reached a pre-determined threshold (e.g., 3% breakthrough of the product), the flow-through from column 1 is directed onto column 2 instead of to the waste (t_1 ; Figure 4). When column 1 is nearly saturated with product and the ΔUV has reached a pre-determined value (t_2 ; Figure 4), the feed is switched to column 2. The column-switching strategy used in PCCSs allows for the uniform loading of the columns irrespective of the feed product concentration and the capacity. Similar switches of the columns based on the level of recombinant protein detected in the eluate from each column can be designed. As in known in the art, column switches can also be designed based on time or the amount of fluid (e.g., buffer) passed through the one or more chromatography column(s) and/or chromatographic membranes in the first or second MCCS.

In PCCSs, the residence time (RT) of the recombinant therapeutic protein on the each chromatography column and/or chromatographic membrane present in the PCCS can be decreased without increasing the column/membrane size because the breakthrough from the first column/membrane can be captured on another column/membrane in the PCCS. A continuous process system can be designed to process liquid culture medium at any perfusion rate (D) by varying the column/membrane volume (V) and RT using the equation of: $V = D * RT$.

The one or more unit operations that can be performed by the at least two MCCSs (e.g., the first and/or second MCCSs) used in the presently described processes include, for example, capturing the recombinant therapeutic protein,

inactivating viruses present in a fluid containing the recombinant therapeutic protein, purifying the recombinant therapeutic protein, polishing the recombinant therapeutic protein, holding a fluid containing the recombinant therapeutic protein (e.g., using any of the exemplary break tank(s) described herein), filtering or removing particulate material and/or cells from a fluid containing the recombinant therapeutic protein, and adjusting the ionic concentration and/or pH of a fluid containing the recombinant therapeutic protein.

The unit operation of capturing can be performed using one or more MCCSs (e.g., a first and/or second MCCS) that includes at least one chromatography column and/or chromatography resin, e.g., that utilizes a capture mechanism. Non-limiting examples of capturing mechanisms include a protein A-binding capture mechanism, an antibody- or antibody fragment-binding capture mechanism, a substrate-binding capture mechanism, an aptamer-binding capture mechanism, a tag-binding capture mechanism (e.g., poly-His tag-based capture mechanism), and a cofactor-binding capture mechanism. Capturing can also be performed using a resin that can be used to perform cation exchange or anion exchange chromatography, or molecular sieve chromatography. Non-limiting resins that can be used to capture a recombinant therapeutic protein are described herein. Additional examples of resins that can be used to capture a recombinant therapeutic protein are known in the art.

The unit operation of inactivating viruses present in a fluid containing the recombinant therapeutic protein can be performed using one or more MCCSs (e.g., a first and/or second MCCS) that include(s), e.g., a chromatography column, a chromatography membrane, or a holding tank that is capable of incubating a fluid containing the recombinant therapeutic protein at a pH of between about 3.0 to 5.0 (e.g., between about 3.5 to about 4.5, between about 3.5 to about 4.25, between about 3.5 to about 4.0, between about 3.5 to about 3.8, or about 3.75) for a period of at least 30 minutes (e.g., a period of between about 30 minutes to 1.5 hours, a period of between about 30 minutes to 1.25 hours, a period of between about 0.75 hours to 1.25 hours, or a period of about 1 hour).

The unit operation of purifying a recombinant protein can be performed using one or more MCCSs (e.g., a first and/or second MCCS) that include(s), e.g., a chromatography column or chromatographic membrane that contains a resin, e.g., that utilizes a capture system. Non-limiting examples of capturing mechanisms include a

protein A-binding capture mechanism, an antibody- or antibody fragment-binding capture mechanism, a substrate-binding capture mechanism, an aptamer-binding capture mechanism, a tag-binding capture mechanism (e.g., poly-His tag-based capture mechanism), and a cofactor-binding capture mechanism. Purifying can also
 5 be performed using a resin that can be used to perform cation exchange or anion exchange chromatography, or molecular sieve chromatography. Non-limiting resins that can be used to purify a recombinant therapeutic protein are described herein. Additional examples of resins that can be used to purify a recombinant therapeutic protein are known in the art.

10 The unit operation of polishing a recombinant protein can be performed using one or more MCCSs (e.g., a first and/or second MCCS) that include(s), e.g., a chromatography column or chromatographic membrane that contains a resin, e.g., that can be used to perform cation exchange, anion exchange, or molecular sieve chromatography. Non-limiting resins that can be used to polish a recombinant
 15 therapeutic protein are described herein. Additional examples of resins that can be used to polish a recombinant therapeutic protein are known in the art.

The unit operation of holding a fluid containing the recombinant therapeutic protein can be performed using an MCCS (e.g., a first and/or second MCCS) that includes at least one reservoir (e.g., a break tank) or a maximum of 1, 2, 3, 4, or 5
 20 reservoir(s) (e.g., break tank(s)) in the first and second MCCS combined. For example, the reservoir(s) (e.g., break tank(s)) that can be used to achieve this unit operation can each have a volume of, e.g., between about 1 mL to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L,
 25 about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about 600 mL, about 500 mL, about 400 mL, about 350 mL, about 300 mL, about 280 mL, about 260 mL, about 240 mL, about 220 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100
 30 mL, about 80 mL, about 60 mL, about 40 mL, about 20 mL, or about 10 mL (inclusive); between about 5 mL to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L, about 600 L, about

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 30 300 L, about 200 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L,
 about 10 L, about 5 L, about 1 L, about 900 mL, about 800 mL, about 600 mL, or
 about 500 mL (inclusive); between about 500 mL to about 10,000 L, about 9,000 L,
 about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about

3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L, about
 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 80 L,
 about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1 L, about 900 mL,
 about 800 mL, or about 600 mL (inclusive); between about 600 mL to about 10,000
 5 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about
 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L,
 about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about
 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, about 1
 L, about 900 mL, or about 800 mL (inclusive); between about 800 mL to about
 10 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L,
 about 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800
 L, about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L,
 about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L,
 about 1 L, or about 900 mL (inclusive); between about 900 mL to about 10,000 L,
 15 about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about
 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L,
 about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about
 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 5 L, or
 about 1 L (inclusive); between about 1 L to about 10,000 L, about 9,000 L, about
 20 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L,
 about 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L, about 600 L,
 about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 80 L, about
 60 L, about 40 L, about 20 L, about 10 L, or about 5 L (inclusive); between about 5 L
 to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about
 25 5,000 L, about 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L,
 about 800 L, about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about
 200 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, or about 10 L
 (inclusive); between about 10 L to about 10,000 L, about 9,000 L, about 8,000 L,
 about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, about
 30 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L, about 600 L, about
 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 80 L, about 60 L,
 about 40 L, or about 20 L (inclusive); between about 20 L to about 10,000 L, about
 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L,

about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L,
 about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about
 80 L, about 60 L, or about 40 L (inclusive); between about 40 L to about 10,000 L,
 about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about
 5 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L,
 about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about
 100 L, about 80 L, or about 60 L (inclusive); between about 60 L to about 10,000 L,
 about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about
 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L,
 10 about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about
 100 L, or about 80 L (inclusive); between about 80 L to about 10,000 L, about 9,000
 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about
 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L, about
 600 L, about 500 L, about 400 L, about 300 L, about 200 L, or about 100 L
 15 (inclusive); between about 100 L to about 10,000 L, about 9,000 L, about 8,000 L,
 about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, about
 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L, about 600 L, about
 500 L, about 400 L, about 300 L, or about 200 L (inclusive); between about 200 L to
 about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about
 20 5,000 L, about 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L,
 about 800 L, about 700 L, about 600 L, about 500 L, about 400 L, or about 300 L
 (inclusive); between about 300 L to about 10,000 L, about 9,000 L, about 8,000 L,
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 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L, about 600 L, about
 25 500 L, or about 400 L (inclusive); between about 400 L to about 10,000 L, about
 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L,
 about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L, about 700 L,
 about 600 L, or about 500 L (inclusive); between about 500 L to about 10,000 L,
 about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about
 30 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L,
 about 700 L, or about 600 L (inclusive); between about 600 L to about 10,000 L,
 about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about
 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, about 800 L, or

about 700 L (inclusive); between about 700 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, about 900 L, or about 800 L (inclusive); between about 800 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, about 2,000 L, about 1,000 L, or about 900 L (inclusive); between about 900 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, about 2,000 L, or about 1,000 L (inclusive); between about 1,000 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, about 3,000 L, or about 2,000 L (inclusive); between about 2,000 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, about 4,000 L, or about 3,000 L (inclusive); between about 3,000 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, about 5,000 L, or about 4,000 L (inclusive); between about 4,000 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, about 6,000 L, or about 5,000 L (inclusive); between about 5,000 L to about 10,000 L, about 9,000 L, about 8,000 L, about 7,000 L, or about 6,000 L (inclusive); between about 6,000 L to about 10,000 L, about 9,000 L, about 8,000 L, or about 7,000 L (inclusive); between about 7,000 L to about 10,000 L, about 9,000 L, or about 8,000 L (inclusive); between about 8,000 L to about 10,000 L or about 9,000 L (inclusive); or between about 9,000 L to about 10,000 L (inclusive).

The reservoir(s) (e.g., break tank(s)) used in the processes described herein can have a capacity that is, e.g., between about 1 mL to about 10,000 L, inclusive (or any of the subranges of this range of volumes described herein). Any of the reservoir(s) (e.g., break tank(s)) used (in any of the processes described herein) to hold fluid before it enters into the first MCCS can have a capacity that is, e.g., between 1 mL and about 100%, inclusive, between about 1 mL and about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about 30%, about 20%, about 10%, or about 5%, inclusive, of the loading volume of the first column of the first MCCS. Any of the reservoir(s) (e.g., break tanks(s)) used to hold a fluid before it enters the second MCCS (and after the fluid leaves the first MCCS) can have a capacity that is, e.g., between 1 mL and about 100%, inclusive, e.g., between about 1 mL and about 90%, about 80%, about 70%, about 60%, about 50%, about 40%, about

30%, about 20%, about 10%, or about 5%, inclusive, of the loading volume of the first column of the second MCCS.

The reservoir(s) (e.g., break tank(s)) can each hold the fluid containing the recombinant therapeutic protein for at least 10 minutes (e.g., at least 20 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, at least 4 hours, at least 6 hours, at least 8 hours, at least 10 hours, at least 12 hours, at least 14 hours, at least 16 hours, at least 18 hours, at least 20 hours, at least 22 hours, at least 24 hours, at least 26 hours, at least 28 hours, at least 30 hours, at least 32 hours, at least 34 hours, at least 36 hours, at least 38 hours, at least 40 hours, at least 42 hours, at least 44 hours, at least 46 hours, or at least 48 hours). In other examples, the reservoir(s) (e.g., break tank(s)) only holds a therapeutic protein for a total time period of, e.g., between about 5 minutes and less than about 48 hours, inclusive, e.g., between about 5 minutes and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, about 30 hours, about 28 hours, about 26 hours, about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, or about 30 minutes, inclusive; between about 30 minutes and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, about 30 hours, about 28 hours, about 26 hours, about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, or about 1 hour, inclusive; between about 1 hour and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, about 30 hours, about 28 hours, about 26 hours, about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, or about 2 hours, inclusive; between about 2 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, about 30 hours, about 28 hours, about 26 hours, about 24 hours, about 22 hours, about 20 hours, about 18

hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours,
 about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, or about 3
 hours, inclusive; between about 3 hours and about 48 hours, about 46 hours, about 44
 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, about 34
 5 hours, about 32 hours, about 30 hours, about 28 hours, about 26 hours, about 24
 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14
 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours,
 about 6 hours, about 5 hours, or about 4 hours, inclusive; between about 4 hours and
 about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about
 10 38 hours, about 36 hours, about 34 hours, about 32 hours, about 30 hours, about 28
 hours, about 26 hours, about 24 hours, about 22 hours, about 20 hours, about 18
 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours,
 about 8 hours, about 7 hours, about 6 hours, or about 5 hours, inclusive; between
 about 5 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours,
 15 about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, about
 30 hours, about 28 hours, about 26 hours, about 24 hours, about 22 hours, about 20
 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10
 hours, about 9 hours, about 8 hours, about 7 hours, or about 6 hours, inclusive;
 between about 6 hours and about 48 hours, about 46 hours, about 44 hours, about 42
 20 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32
 hours, about 30 hours, about 28 hours, about 26 hours, about 24 hours, about 22
 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12
 hours, about 10 hours, about 9 hours, about 8 hours, or about 7 hours, inclusive;
 between about 7 hours and about 48 hours, about 46 hours, about 44 hours, about 42
 25 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32
 hours, about 30 hours, about 28 hours, about 26 hours, about 24 hours, about 22
 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12
 hours, about 10 hours, about 9 hours, or about 8 hours, inclusive; between about 8
 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40
 30 hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, about 30
 hours, about 28 hours, about 26 hours, about 24 hours, about 22 hours, about 20
 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10
 hours, or about 9 hours, inclusive; between about 9 hours and about 48 hours, about

[illegible]

hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, about 30 hours, or about 28 hours, inclusive; between about 28 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, about 32 hours, or about 30 hours, inclusive; between about 30 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, about 34 hours, or about 32 hours, inclusive; between about 32 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, about 36 hours, or about 34 hours, inclusive; between about 34 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, about 38 hours, or about 36 hours, inclusive; between about 36 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, about 40 hours, or about 38 hours, inclusive; between about 38 hours and about 48 hours, about 46 hours, about 44 hours, about 42 hours, or about 40 hours, inclusive; between about 40 hours and about 48 hours, about 46 hours, about 44 hours, or about 42 hours, inclusive; between about 42 hours and about 48 hours, about 46 hours, or about 44 hours, inclusive; between about 44 hours and about 48 hours or about 46 hours, inclusive; between about 46 hours and about 48 hours, inclusive.

The reservoir(s) (e.g., break tank(s)) can be used to both hold and refrigerate (e.g., at a temperature of less than 25 °C, less than 15 °C, or less than 10 °C) the fluid containing the recombinant therapeutic protein. The reservoir can have any shape, including a circular cylinder, an oval cylinder, or an approximately rectangular sealed and nonpermeable bag.

The unit operations of filtering a fluid containing the recombinant therapeutic protein can be performed using an MCCS (e.g., the first and/or second MCCS) that includes, e.g., a filter, or a chromatography column or chromatographic membrane that contains a molecule sieve resin. As is known in the art, a wide variety of submicron filters (e.g., a filter with a pore size of less than 1 μm , less than 0.5 μm , less than 0.3 μm , about 0.2 μm , less than 0.2 μm , less than 100 nm, less than 80 nm, less than 60 nm, less than 40 nm, less than 20 nm, or less than 10 nm) are available in the art that are capable of removing any precipitated material and/or cells (e.g., precipitated, unfolded protein; precipitated, unwanted host cell proteins; precipitated lipids; bacteria; yeast cells; fungal cells; mycobacteria; and/or mammalian cells). Filters having a pore size of about 0.2 μm or less than 0.2 μm are known to effectively

remove bacteria from the fluid containing the recombinant therapeutic protein. As is known in the art, a chromatography column or a chromatographic membrane containing a molecular sieve resin can also be used in an MCCS (e.g., the first and/or second MCCS) to perform the unit operation of filtering a fluid containing a recombinant therapeutic protein.

The unit operations of adjusting the ionic concentration and/or pH of a fluid containing the recombinant therapeutic protein can be performed using a MCCS (e.g., the first and/or second MCCS) that includes and utilizes a buffer adjustment reservoir (e.g., an in-line buffer adjustment reservoir) that adds a new buffer solution into a fluid that contains the recombinant therapeutic protein (e.g., between columns within a single MCCS, or after the last column in a penultimate MCCS (e.g., the first MCCS) and before the fluid containing the recombinant therapeutic protein is fed into the first column of the next MCCS (e.g., the second MCCS). As can be appreciated in the art, the in-line buffer adjustment reservoir can be any size (e.g., greater than 100 mL) and can contain any buffered solution (e.g., a buffered solution that has one or more of: an increased or decreased pH as compared to the fluid containing the recombinant therapeutic protein, a an increased or decreased ionic (e.g., salt) concentration compared to the fluid containing the recombinant therapeutic protein, and/or an increased or decreased concentration of an agent that competes with the recombinant therapeutic protein for binding to resin present in at least one chromatographic column or at least one chromatographic membrane in an MCCS (e.g., the first or the second MCCS)). In other examples, the unit operations of adjusting the ionic concentration and/or pH of a fluid can be performed using size exclusion chromatography and counter-current dialysis (see, e.g., Kurnik et al., *Biotechnol. Bioeng.* 45(2):149-157, 1995; and Klutz et al., *J. Biotechnol.* 213:120-130, 2015).

The first and/or second MCCS can perform two or more unit operations. For example, the first and/or second MCCS can each perform at least the following unit operations: capturing the recombinant therapeutic protein and inactivating viruses present in the fluid containing the recombinant therapeutic protein; capturing the recombinant therapeutic protein, inactivating viruses present in the fluid containing the recombinant therapeutic protein, and adjusting the ionic concentration and/or pH of a liquid containing the recombinant therapeutic protein; purifying the recombinant therapeutic protein and polishing the recombinant therapeutic protein;

purifying the recombinant therapeutic protein, polishing the recombinant therapeutic protein, and filtering a fluid containing the recombinant therapeutic protein or removing precipitates and/or particular matter from a fluid containing the recombinant therapeutic protein; and purifying the recombinant therapeutic protein, polishing the recombinant therapeutic protein, filtering a fluid containing the recombinant therapeutic protein or removing precipitates and/or particular matter from a fluid containing the recombinant therapeutic protein, and adjusting the ionic concentration and/or pH of a liquid containing the recombinant therapeutic protein.

10 ***Capturing the Recombinant Therapeutic Protein***

The present processes include a step of capturing the recombinant therapeutic protein using a first MCCS. As can be appreciated in the art, the liquid culture medium containing the recombinant therapeutic protein can be continuously fed onto the first MCCS using a variety of different means. For example, the liquid culture medium can be actively pumped into the first MCCS or the liquid culture medium can be fed into the first MCCS using gravitational force. The liquid culture medium can be stored in a reservoir (e.g., a holding tank) before it is fed into the first MCCS or the liquid culture medium can be actively pumped from a bioreactor containing a culture of cells (e.g., mammalian cells that secrete the recombinant therapeutic protein into the culture medium) into the first MCCS.

The liquid culture medium can be fed (loaded) into the first MCCS at a flow rate of, e.g., between about 0.2 mL/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute, about 95 mL/minute, about 90 mL/minute, about 85 mL/minute, about 80 mL/minute, about 75 mL/minute, about

70 mL/minute, about 65 mL/minute, about 60 mL/minute, about 55 mL/minute, about
 50 mL/minute, about 45 mL/minute, about 40 mL/minute, about 35 mL/minute, about
 30 mL/minute, about 25 mL/minute, about 20 mL/minute, about 15 mL/minute, about
 10 mL/minute, about 5 mL/minute, or about 2 mL/minute (inclusive); between about
 5 0.5 mL/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about
 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65
 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45
 L/minute, about 40 L/minute, about 35 L/minute, about 30 L/minute, about 25
 L/minute, about 20 L/minute, about 15 L/minute, about 10 L/minute, about 5
 10 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850
 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute,
 about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500
 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute,
 about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150
 15 mL/minute, about 100 mL/minute, about 95 mL/minute, about 90 mL/minute, about
 85 mL/minute, about 80 mL/minute, about 75 mL/minute, about 70 mL/minute, about
 65 mL/minute, about 60 mL/minute, about 55 mL/minute, about 50 mL/minute, about
 45 mL/minute, about 40 mL/minute, about 35 mL/minute, about 30 mL/minute, about
 25 mL/minute, about 20 mL/minute, about 15 mL/minute, about 10 mL/minute, about
 20 5 mL/minute, or about 2 mL/minute (inclusive); between about 1 mL/minute to about
 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80
 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60
 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40
 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20
 25 L/minute, about 15 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
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 mL/minute, about 750 mL/minute, about 700 mL/minute, about 650 mL/minute,
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 30 about 250 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100
 mL/minute, about 95 mL/minute, about 90 mL/minute, about 85 mL/minute, about 80
 mL/minute, about 75 mL/minute, about 70 mL/minute, about 65 mL/minute, about 60
 mL/minute, about 55 mL/minute, about 50 mL/minute, about 45 mL/minute, about 40

mL/minute, about 35 mL/minute, about 30 mL/minute, about 25 mL/minute, about 20
 mL/minute, about 15 mL/minute, about 10 mL/minute, about 5 mL/minute, or about 2
 mL/minute (inclusive); between about 2 mL/minute to about 100 L/minute, about 95
 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75
 5 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55
 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35
 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 10 about 750 mL/minute, about 700 mL/minute, about 650 mL/minute, about 600
 mL/minute, about 550 mL/minute, about 500 mL/minute, about 450 mL/minute,
 about 400 mL/minute, about 350 mL/minute, about 300 mL/minute, about 250
 mL/minute, about 200 mL/minute, about 150 mL/minute, about 100 mL/minute,
 about 95 mL/minute, about 90 mL/minute, about 85 mL/minute, about 80 mL/minute,
 15 about 75 mL/minute, about 70 mL/minute, about 65 mL/minute, about 60 mL/minute,
 about 55 mL/minute, about 50 mL/minute, about 45 mL/minute, about 40 mL/minute,
 about 35 mL/minute, about 30 mL/minute, about 25 mL/minute, about 20 mL/minute,
 about 15 mL/minute, about 10 mL/minute, or about 5 mL/minute (inclusive); between
 about 5 mL/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute,
 20 about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about
 65 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45
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 L/minute, about 20 L/minute, about 15 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 950 mL/minute, about 900 mL/minute, about 850
 25 mL/minute, about 800 mL/minute, about 750 mL/minute, about 700 mL/minute,
 about 650 mL/minute, about 600 mL/minute, about 550 mL/minute, about 500
 mL/minute, about 450 mL/minute, about 400 mL/minute, about 350 mL/minute,
 about 300 mL/minute, about 250 mL/minute, about 200 mL/minute, about 150
 mL/minute, about 100 mL/minute, about 95 mL/minute, about 90 mL/minute, about
 30 85 mL/minute, about 80 mL/minute, about 75 mL/minute, about 70 mL/minute, about
 65 mL/minute, about 60 mL/minute, about 55 mL/minute, about 50 mL/minute, about
 45 mL/minute, about 40 mL/minute, about 35 mL/minute, about 30 mL/minute, about
 25 mL/minute, about 20 mL/minute, about 15 mL/minute, or about 10 mL/minute

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 mL/minute (inclusive); between about 600 mL/minute to about 100 L/minute, about
 30 95 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75
 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55
 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35
 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15

L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800 mL/minute,
 about 750 mL/minute, about 700 mL/minute, or about 650 mL/minute (inclusive);
 between about 650 mL/minute to about 100 L/minute, about 95 L/minute, about 90
 5 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70
 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50
 L/minute, about 45 L/minute, about 40 L/minute, about 35 L/minute, about 30
 L/minute, about 25 L/minute, about 20 L/minute, about 15 L/minute, about 10
 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, about 900
 10 mL/minute, about 850 mL/minute, about 800 mL/minute, about 750 mL/minute, or
 about 700 mL/minute (inclusive); between about 700 mL/minute to about 100
 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80
 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60
 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40
 15 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20
 L/minute, about 15 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, about 800
 mL/minute, or about 750 mL/minute (inclusive); between about 750 mL/minute to
 about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about
 20 80 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60
 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40
 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20
 L/minute, about 15 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 950 mL/minute, about 900 mL/minute, about 850 mL/minute, or about 800
 25 mL/minute (inclusive); between about 800 mL/minute to about 100 L/minute, about
 95 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75
 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55
 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35
 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15
 30 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950
 mL/minute, about 900 mL/minute, or about 850 mL/minute (inclusive); between
 about 850 mL/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute,
 about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about

65 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 950 mL/minute, or about 900 mL/minute (inclusive); between about 900 mL/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, or about 950 mL/minute (inclusive); between about 950 mL/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15 L/minute, about 10 L/minute, about 5 L/minute, or about 1 L/minute (inclusive); between about 1 L/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15 L/minute, or about 10 L/minute (inclusive); between about 10 L/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, about 20 L/minute, about 15 L/minute, or about 10 L/minute (inclusive); between about 15 L/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80

L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60
 L/minute, about 55 L/minute, about 50 L/minute, about 45 L/minute, about 40
 L/minute, about 35 L/minute, about 30 L/minute, about 25 L/minute, or about 20
 L/minute (inclusive); between about 20 L/minute to about 100 L/minute, about 95
 5 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75
 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55
 L/minute, about 50 L/minute, about 45 L/minute, about 40 L/minute, about 35
 L/minute, about 30 L/minute, or about 25 L/minute (inclusive); between about 25
 L/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85
 10 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65
 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, about 45
 L/minute, about 40 L/minute, about 35 L/minute, or about 30 L/minute (inclusive);
 between about 30 L/minute to about 100 L/minute, about 95 L/minute, about 90
 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70
 15 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50
 L/minute, about 45 L/minute, about 40 L/minute, or about 35 L/minute (inclusive);
 between about 35 L/minute to about 100 L/minute, about 95 L/minute, about 90
 L/minute, about 85 L/minute, about 80 L/minute, about 75 L/minute, about 70
 L/minute, about 65 L/minute, about 60 L/minute, about 55 L/minute, about 50
 20 L/minute, about 45 L/minute, or about 40 L/minute (inclusive); between about 40
 L/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85
 L/minute, about 80 L/minute, about 75 L/minute, about 70 L/minute, about 65
 L/minute, about 60 L/minute, about 55 L/minute, about 50 L/minute, or about 45
 L/minute (inclusive); between about 45 L/minute to about 100 L/minute, about 95
 25 L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75
 L/minute, about 70 L/minute, about 65 L/minute, about 60 L/minute, about 55
 L/minute, or about 50 L/minute (inclusive); between about 50 L/minute to about 100
 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80
 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, about 60
 30 L/minute, or about 55 L/minute (inclusive); between about 55 L/minute to about 100
 L/minute, about 95 L/minute, about 90 L/minute, about 85 L/minute, about 80
 L/minute, about 75 L/minute, about 70 L/minute, about 65 L/minute, or about 60
 L/minute (inclusive); between about 60 L/minute to about 100 L/minute, about 95

L/minute, about 90 L/minute, about 85 L/minute, about 80 L/minute, about 75
 L/minute, about 70 L/minute, or about 65 L/minute (inclusive); between about 65
 L/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, about 85
 L/minute, about 80 L/minute, about 75 L/minute, or about 70 L/minute (inclusive);
 5 between about 70 L/minute to about 100 L/minute, about 95 L/minute, about 90
 L/minute, about 85 L/minute, about 80 L/minute, or about 75 L/minute (inclusive);
 between about 75 L/minute to about 100 L/minute, about 95 L/minute, about 90
 L/minute, about 85 L/minute, or about 80 L/minute (inclusive); between about 80
 L/minute to about 100 L/minute, about 95 L/minute, about 90 L/minute, or about 85
 10 L/minute (inclusive); between about 85 L/minute to about 100 L/minute, about 95
 L/minute, or about 90 L/minute (inclusive); between about 90 L/minute to about 100
 L/minute or about 95 L/minute (inclusive); or about 95 L/minute to about 100
 L/minute (inclusive). The liquid culture medium containing the recombinant
 therapeutic protein can be derived from any of the exemplary sources described herein
 15 or known in the art.

Some examples further include the optional step of filtering the liquid culture
 medium before it is fed onto the first MCCS. Any of the exemplary means of filtering
 a liquid culture medium or a fluid containing the recombinant therapeutic protein
 described herein, or any filtration means known in the art, can be used to filter the
 20 liquid culture medium containing the recombinant therapeutic protein before it is fed
 into the first MCCS.

In the methods described herein, the capturing of the recombinant therapeutic
 protein from the liquid culture medium is performed using a first MCCS. As can be
 appreciated in the art, in order to achieve the capture of the recombinant therapeutic
 25 protein, at least one chromatographic column or at least one chromatographic
 membrane in the first MCCS must contain a resin that utilizes a capturing mechanism
 (e.g., any of the exemplary capturing mechanisms described herein), or contains a
 resin capable of performing cation exchange, anion exchange, or molecule sieve
 chromatography. For example, if the recombinant therapeutic protein is an antibody
 30 or an antibody fragment, the capturing system can be a protein A-binding capturing
 mechanism or an antigen-binding capturing mechanism (where the capturing antigen
 is specifically recognized by the recombinant therapeutic antibody or antibody
 fragment). If the recombinant therapeutic protein is an enzyme, the capturing

mechanism can use an antibody or antibody fragment that specifically binds to the enzyme to capture the recombinant therapeutic enzyme, a substrate of the enzyme to capture the recombinant therapeutic enzyme, a cofactor of the enzyme to capture the recombinant therapeutic enzyme, or, if the recombinant therapeutic enzyme contains a tag, a protein, metal chelate, or antibody (or antibody fragment) that specifically binds to the tag present in the recombinant therapeutic enzyme. Non-limiting resins that can be used to capture a recombinant therapeutic protein are described herein and additional resins that can be used to capture a recombinant therapeutic protein are known in the art. One non-limiting example of resin that utilizes a protein A-binding capture mechanism is MabSelect SuRe resin (GE Healthcare, Piscataway, NJ).

Exemplary non-limiting sizes and shapes of the chromatography column(s) or chromatographic membrane(s) present in the first MCCS that can be used to capture the recombinant therapeutic protein are described herein. The liquid culture medium fed (loaded) into the first MCCS can contain, e.g., between about 0.05 mg/mL to about 100 mg/mL recombinant therapeutic protein (e.g., between about 0.1 mg/mL to about 90 mg/mL, between about 0.1 mg/mL to about 80 mg/mL, between about 0.1 mg/mL to about 70 mg/mL, between about 0.1 mg/mL to about 60 mg/mL, between about 0.1 mg/mL to about 50 mg/mL, between about 0.1 mg/mL to about 40 mg/mL, between about 0.1 mg/mL to about 30 mg/mL, between about 0.1 mg/mL to about 20 mg/mL, between 0.5 mg/mL to about 20 mg/mL, between about 0.1 mg/mL to about 15 mg/mL, between about 0.5 mg/mL to about 15 mg/mL, between about 0.1 mg/mL to about 10 mg/mL, or between about 0.5 mg/mL to about 10 mg/mL recombinant therapeutic protein). The mean time required for the recombinant therapeutic protein to bind to the resin used to perform the unit operation of capturing can be, e.g., between about 1 second to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, about 18 minutes, about 16 minutes, about 14 minutes, about 12 minutes, about 10 minutes, about 8 minutes, about 6 minutes, about 5 minutes, about 4 minutes, about 3 minutes, about 2 minutes, about 1 minute, about 30 seconds, about 20 seconds, about 10 seconds, or about 5 seconds (inclusive); between about 5 seconds to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours,

about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40
 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes,
 about 18 minutes, about 16 minutes, about 14 minutes, about 12 minutes, about 10
 minutes, about 8 minutes, about 6 minutes, about 5 minutes, about 4 minutes, about 3
 5 minutes, about 2 minutes, about 1 minute, about 30 seconds, about 20 seconds, or
 about 10 seconds (inclusive); between about 10 seconds to about 24 hours, about 20
 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours,
 about 2 hours, about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes,
 about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20
 10 minutes, about 18 minutes, about 16 minutes, about 14 minutes, about 12 minutes,
 about 10 minutes, about 8 minutes, about 6 minutes, about 5 minutes, about 4
 minutes, about 3 minutes, about 2 minutes, about 1 minute, about 30 seconds, or
 about 20 seconds (inclusive); between about 20 seconds to about 24 hours, about 20
 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours,
 15 about 2 hours, about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes,
 about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20
 minutes, about 18 minutes, about 16 minutes, about 14 minutes, about 12 minutes,
 about 10 minutes, about 8 minutes, about 6 minutes, about 5 minutes, about 4
 minutes, about 3 minutes, about 2 minutes, about 1 minute, or about 30 seconds
 20 (inclusive); between about 30 seconds to about 24 hours, about 20 hours, about 16
 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours,
 about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40
 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes,
 about 18 minutes, about 16 minutes, about 14 minutes, about 12 minutes, about 10
 25 minutes, about 8 minutes, about 6 minutes, about 5 minutes, about 4 minutes, about 3
 minutes, about 2 minutes, or about 1 minute (inclusive); between about 1 minute to
 about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about
 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55 minutes, about 50
 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes,
 30 about 25 minutes, about 20 minutes, about 18 minutes, about 16 minutes, about 14
 minutes, about 12 minutes, about 10 minutes, about 8 minutes, about 6 minutes, about
 5 minutes, about 4 minutes, about 3 minutes, or about 2 minutes (inclusive); between
 about 2 minutes to about 24 hours, about 20 hours, about 16 hours, about 12 hours,

about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55
 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
 about 30 minutes, about 25 minutes, about 20 minutes, about 18 minutes, about 16
 minutes, about 14 minutes, about 12 minutes, about 10 minutes, about 8 minutes,
 5 about 6 minutes, about 5 minutes, about 4 minutes, or about 3 minutes (inclusive);
 between about 3 minutes to about 24 hours, about 20 hours, about 16 hours, about 12
 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about
 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
 about 30 minutes, about 25 minutes, about 20 minutes, about 18 minutes, about 16
 10 minutes, about 14 minutes, about 12 minutes, about 10 minutes, about 8 minutes,
 about 6 minutes, about 5 minutes, or about 4 minutes (inclusive); between about 4
 minutes to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8
 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55 minutes,
 about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30
 15 minutes, about 25 minutes, about 20 minutes, about 18 minutes, about 16 minutes,
 about 14 minutes, about 12 minutes, about 10 minutes, about 8 minutes, about 6
 minutes, or about 5 minutes (inclusive); between about 5 minutes to about 24 hours,
 about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3
 hours, about 2 hours, about 1 hour, about 55 minutes, about 50 minutes, about 45
 20 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes,
 about 20 minutes, about 18 minutes, about 16 minutes, about 14 minutes, about 12
 minutes, about 10 minutes, about 8 minutes, or about 6 minutes (inclusive); between
 about 6 minutes to about 24 hours, about 20 hours, about 16 hours, about 12 hours,
 about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55
 25 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
 about 30 minutes, about 25 minutes, about 20 minutes, about 18 minutes, about 16
 minutes, about 14 minutes, about 12 minutes, about 10 minutes, or about 8 minutes
 (inclusive); between about 8 minutes to about 60 minutes, about 55 minutes, about 50
 minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes,
 30 about 25 minutes, about 20 minutes, about 18 minutes, about 16 minutes, about 14
 minutes, about 12 minutes, or about 10 minutes (inclusive); between about 10 minutes
 to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours,
 about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55 minutes, about 50

minutes, about 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes,
 about 25 minutes, about 20 minutes, about 18 minutes, about 16 minutes, about 14
 minutes, or about 12 minutes (inclusive); between about 12 minutes to about 24
 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours,
 5 about 3 hours, about 2 hours, about 1 hour, about 55 minutes, about 50 minutes, about
 45 minutes, about 40 minutes, about 35 minutes, about 30 minutes, about 25 minutes,
 about 20 minutes, about 18 minutes, about 16 minutes, or about 14 minutes
 (inclusive); between about 14 minutes to about 24 hours, about 20 hours, about 16
 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours,
 10 about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40
 minutes, about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes,
 about 18 minutes, or about 16 minutes (inclusive); between about 16 minutes to about
 60 minutes, about 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes,
 about 35 minutes, about 30 minutes, about 25 minutes, about 20 minutes, or about 18
 15 minutes (inclusive); between about 18 minutes to about 24 hours, about 20 hours,
 about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2
 hours, about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40
 minutes, about 35 minutes, about 30 minutes, about 25 minutes, or about 20 minutes
 (inclusive); between about 20 minutes to about 24 hours, about 20 hours, about 16
 20 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours,
 about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes, about 40
 minutes, about 35 minutes, about 30 minutes, or about 25 minutes (inclusive);
 between about 25 minutes to about 24 hours, about 20 hours, about 16 hours, about 12
 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about
 25 55 minutes, about 50 minutes, about 45 minutes, about 40 minutes, about 35 minutes,
 or about 30 minutes (inclusive); between about 30 minutes to about 24 hours, about
 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours,
 about 2 hours, about 1 hour, about 55 minutes, about 50 minutes, about 45 minutes,
 about 40 minutes, or about 35 minutes (inclusive); between about 35 minutes to about
 30 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4
 hours, about 3 hours, about 2 hours, about 1 hour, about 55 minutes, about 50
 minutes, about 45 minutes, or about 40 minutes (inclusive); between about 40 minutes
 to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours,

about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55 minutes, about 50 minutes, or about 45 minutes (inclusive); between about 45 minutes to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 55 minutes, or about 50 minutes (inclusive); between about 50 minutes to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, or about 55 minutes (inclusive); about 55 minutes to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, or about 60 minutes (inclusive); between about 60 minutes to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, about 2 hours, or about 1 hour (inclusive); between about 1 hour to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 3 hours, or about 2 hours (inclusive); between about 2 hours to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, or about 3 hours (inclusive); between about 3 hours to about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, or about 4 hours (inclusive); between about 4 hours to about 24 hours, about 20 hours, about 16 hours, about 12 hours, or about 8 hours (inclusive); between about 8 hours to about 24 hours, about 20 hours, about 16 hours, or about 12 hours (inclusive); between about 12 hours to about 24 hours, about 20 hours, or about 16 hours (inclusive); between about 16 hours to about 24 hours or about 20 hours (inclusive); or between about 20 hours to about 24 hours (inclusive).

As can be appreciated in the art, in order to capture the recombinant therapeutic protein using the chromatography column(s) or chromatographic membrane(s) present in the first MCCS, one must perform the sequential chromatographic steps of loading, washing, eluting, and regenerating the chromatography column(s) or chromatography membrane(s) present in the first MCCS. Any of the exemplary flow rates, buffer volumes, and/or lengths of time allotted for each sequential chromatographic step described herein can be used in the one or more of these different sequential chromatographic steps (e.g., one or more of the sequential chromatographic steps of loading, washing, eluting, and regenerating the chromatography column(s) or chromatography membrane(s) present in the first MCCS that are used for capturing the recombinant therapeutic protein). Non-limiting

flow rates, buffer volumes, and/or lengths of time allotted for each sequential chromatographic step that can be used for capturing chromatographic column(s) and/or chromatographic membrane(s) in the first MCCS (e.g., a first PCC system) are provided below. In addition, exemplary buffers elution buffers that can be used in the first MCCS are described below.

The first MCCS containing at least one chromatographic column and/or chromatographic membrane containing a resin that can perform the unit operation of capturing (e.g., any of exemplary resins that can be used for capturing described herein) can be loaded with the liquid culture medium containing a recombinant therapeutic protein using any of loading flow rates (fed rates) described above. In some examples, a single chromatographic column or single chromatographic membrane containing a resin that is capable of performing the unit operation of capturing is loaded in, e.g., between about 10 minutes to about 48 hours, about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 110 minutes, about 100 minutes, about 90 minutes, about 80 minutes, about 70 minutes, about 60 minutes, about 50 minutes, about 40 minutes, about 30 minutes, about 20 minutes, or about 15 minutes (inclusive); between about 15 minutes to about 48 hours, about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 110 minutes, about 100 minutes, about 90 minutes, about 80 minutes, about 70 minutes, about 60 minutes, about 50 minutes, about 40 minutes, about 30 minutes, or about 20 minutes (inclusive); between about 20 minutes to about 48 hours, about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 110 minutes, about 100 minutes, about 90 minutes, about 80 minutes, about 70 minutes, about 60 minutes, about 50 minutes, about 40 minutes, or about 30 minutes (inclusive); between about 30 minutes to about 48 hours, about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 7 hours, about 6 hours,

about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 110 minutes, about
 100 minutes, about 90 minutes, about 80 minutes, about 70 minutes, about 60
 minutes, about 50 minutes, or about 40 minutes (inclusive); between about 40 minutes
 to about 48 hours, about 44 hours, about 40 hours, about 36 hours, about 32 hours,
 5 about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about
 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours,
 about 2 hours, about 110 minutes, about 100 minutes, about 90 minutes, about 80
 minutes, about 70 minutes, about 60 minutes, or about 50 minutes (inclusive);
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 10 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16
 hours, about 12 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours,
 about 4 hours, about 3 hours, about 2 hours, about 110 minutes, about 100 minutes,
 about 90 minutes, about 80 minutes, about 70 minutes, or about 60 minutes
 (inclusive); between about 60 minutes to about 48 hours, about 44 hours, about 40
 15 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20
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 about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 110 minutes, about
 100 minutes, about 90 minutes, about 80 minutes, or about 70 minutes (inclusive);
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 about 4 hours, about 3 hours, about 2 hours, about 110 minutes, about 100 minutes,
 about 90 minutes, or about 80 minutes (inclusive); between about 80 minutes to about
 48 hours, about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28
 25 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours,
 about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2
 hours, about 110 minutes, about 100 minutes, or about 90 minutes (inclusive);
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 12 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, or
 10 about 3 hours (inclusive); between about 3 hours to about 48 hours, about 44 hours,
 about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about
 20 hours, about 16 hours, about 12 hours, about 8 hours, about 7 hours, about 6 hours,
 about 5 hours, or about 4 hours (inclusive); between about 4 hours to about 48 hours,
 about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about
 15 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 7
 hours, about 6 hours, or about 5 hours (inclusive); between about 5 hours to about 48
 hours, about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28
 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours,
 about 7 hours, or about 6 hours (inclusive); between about 6 hours to about 48 hours,
 20 about 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about
 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, or about 7
 hours (inclusive); between about 7 hours to about 48 hours, about 44 hours, about 40
 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20
 hours, about 16 hours, about 12 hours, or about 8 hours (inclusive); between about 8
 25 hours to about 48 hours, about 44 hours, about 40 hours, about 36 hours, about 32
 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, or about 12
 hours (inclusive); between about 12 hours to about 48 hours, about 44 hours, about 40
 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20
 hours, or about 16 hours (inclusive); between about 16 hours to about 48 hours, about
 30 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24
 hours, or about 20 hours (inclusive); between about 20 hours to about 48 hours, about
 44 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, or about 24
 hours (inclusive); between about 24 hours to about 48 hours, about 44 hours, about 40

hours, about 36 hours, about 32 hours, or about 28 hours (inclusive); between about 28 hours to about 48 hours, about 44 hours, about 40 hours, about 36 hours, or about 32 hours (inclusive); between about 32 hours to about 48 hours, about 44 hours, about 40 hours, or about 36 hours (inclusive); between about 36 hours to about 48 hours, about 44 hours, or about 40 hours (inclusive); between about 40 hours to about 48 hours or about 44 hours (inclusive), or about 44 hours to about 48 hours (inclusive). In some examples, wherein the first MCCS includes at least two chromatographic columns that contain a resin that is capable of performing the unit operation of capturing in series, the time required to load two of the chromatographic columns in series is, e.g., between about 50 minutes to about 180 minutes (e.g., between about 60 minutes and about 180 minutes, between about 70 minutes and about 180 minutes, between about 80 minutes and about 180 minutes, between about 90 minutes and about 180 minutes, between about 100 minutes and about 180 minutes, between about 110 minutes and 150 minutes, and between about 125 minutes and about 145 minutes).

Following the loading of the recombinant therapeutic protein onto the at least one chromatographic column or chromatographic membrane in the first MCCS that contains a resin that is capable of performing the unit operation of capturing, the at least one chromatographic column or chromatographic membrane is washed with at least one washing buffer. As can be appreciated in the art, the at least one (e.g., two, three, or four) washing buffer is meant to elute all proteins that are not the recombinant therapeutic protein from the at least one chromatography column or chromatographic membrane, while not disturbing the interaction of the recombinant therapeutic protein with the resin.

The wash buffer can be passed through the at least one chromatography column or chromatographic membrane at a flow rate of, e.g., between about 0.2 mL/minute to about 100 L/minute (inclusive) (e.g., any of the subranges of this flow rate range described herein). The volume of wash buffer used (e.g., combined total volume of wash buffer used when more than one wash buffer is used) can be, e.g., between about 1X column volume (CV) to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, about 4X CV, or

about 3X CV (inclusive); between about 2X CV to about 30X CV, about 28X CV,
 about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about
 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X
 CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, or about
 5 4X CV (inclusive); between about 3X CV to about 30X CV, about 28X CV, about
 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X
 CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV,
 about 9X CV, about 8X CV, about 7X CV, or about 5X CV (inclusive); between
 about 4X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about
 10 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X
 CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV,
 about 7X CV, or about 6X CV (inclusive); between about 5X CV to about 30X CV,
 about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about
 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X
 15 CV, about 10X CV, about 9X CV, about 8X CV, or about 7X CV (inclusive);
 between about 6X CV to about 30X CV, about 28X CV, about 26X CV, about 24X
 CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV,
 about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, or about
 8X CV (inclusive); between about 7X CV to about 30X CV, about 28X CV, about
 20 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X
 CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, or
 about 9X CV (inclusive); between about 8X CV to about 30X CV, about 28X CV,
 about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about
 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, or about 10X
 25 CV (inclusive); between about 9 CV to about 30X CV, about 28X CV, about 26X
 CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV,
 about 14X CV, about 13X CV, about 12X CV, or about 11X CV (inclusive); between
 about 10X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV,
 about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about
 30 13X CV, or about 12X CV (inclusive); between about 11X CV to about 30X CV,
 about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about
 18X CV, about 16X CV, about 14X CV, or about 13X CV (inclusive); between about
 12X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X

CV, about 20X CV, about 18X CV, about 16X CV, or about 14X CV (inclusive);
 between about 14X CV to about 30X CV, about 28X CV, about 26X CV, about 24X
 CV, about 22X CV, about 20X CV, about 18X CV, or about 16X CV (inclusive);
 between about 16X CV to about 30X CV, about 28X CV, about 26X CV, about 24X
 5 CV, about 22X CV, about 20X CV, or about 18X CV (inclusive); between about 18X
 CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV,
 or about 20X CV (inclusive); between about 20X CV to about 30X CV, about 28X
 CV, about 26X CV, about 24X CV, or about 22X CV (inclusive); between about 22X
 CV to about 30X CV, about 28X CV, about 26X CV, or about 24X CV (inclusive);
 10 between about 24X CV to about 30X CV, about 28X CV, or about 26X CV
 (inclusive); between about 26X CV to about 30X CV or about 28X CV (inclusive); or
 between about 28X CV to about 30X CV (inclusive).

The total time of the washing can be, e.g., between about 2 minutes to about
 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14
 15 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours,
 about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3
 hours, about 2.5 hours, about 2 hours, about 1.5 hours, about 1 hour, about 50
 minutes, about 40 minutes, about 30 minutes, about 20 minutes, about 10 minutes, or
 about 5 minutes (inclusive); between about 5 minutes to about 24 hours, about 22
 20 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12
 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours,
 about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, about
 2.5 hours, about 2 hours, about 1.5 hours, about 1 hour, about 50 minutes, about 40
 minutes, about 30 minutes, about 20 minutes, or about 10 minutes (inclusive); about
 25 10 minutes to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about
 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8
 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours,
 about 3.5 hours, about 3 hours, about 2.5 hours, about 2 hours, about 1.5 hours, about
 1 hour, about 50 minutes, about 40 minutes, about 30 minutes, or about 20 minutes
 30 (inclusive); between about 20 minutes to about 24 hours, about 22 hours, about 20
 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10
 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours,
 about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, about 2.5 hours, about

2 hours, about 1.5 hours, about 1 hour, about 50 minutes, about 40 minutes, or about 30 minutes (inclusive); between about 30 minutes to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, about 2.5 hours, about 2 hours, about 1.5 hours, about 1 hour, about 50 minutes, or about 40 minutes (inclusive); between about 40 minutes to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, about 2.5 hours, about 2 hours, about 1.5 hours, about 1 hour, or about 50 minutes (inclusive); between about 50 minutes to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, about 2.5 hours, about 2 hours, about 1.5 hours, or about 1 hour (inclusive); between about 1 hour to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, about 2.5 hours, about 2 hours, or about 1.5 hours (inclusive); between about 1.5 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, about 2.5 hours, or about 2 hours (inclusive); between about 2 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, about 3 hours, or about 2.5 hours (inclusive); between about 2.5 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, about 3.5 hours, or about 3 hours (inclusive); between about 3 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about

8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, about 4 hours, or about 3.5 hours (inclusive); between about 3.5 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4.5 hours, or about 4 hours (inclusive); between about 4 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, about 6 hours, about 5 hours, or about 4.5 hours (inclusive); between about 4.5 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, about 8 hours, about 7 hours, or about 6 hours (inclusive); between about 6 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 9 hours, or about 8 hours (inclusive); between about 8 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, or about 9 hours (inclusive); between about 9 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, or about 10 hours (inclusive); between about 10 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, or about 12 hours (inclusive); between about 12 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, or about 14 hours (inclusive); between about 14 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, or about 16 hours (inclusive); between about 16 hours to about 24 hours, about 22 hours, about 20 hours, or about 18 hours (inclusive); between about 18 hours to about 24 hours, about 22 hours, or about 20 hours (inclusive); between about 20 hours to about 24 hours or about 22 hours (inclusive); or between about 22 hours to about 24 hours (inclusive).

Following the washing of the at least one chromatographic column or chromatographic membrane in the first MCCS that contains a resin that is capable of performing the unit operation of capturing, the recombinant therapeutic protein is eluted from the at least one chromatographic column or chromatographic membrane by passing an elution buffer through the at least one chromatographic column or chromatographic membrane in the first MCCS that contains a resin that is capable of performing the unit operation of capturing. The elution buffer can be passed through the at least one chromatography column or chromatographic membrane that contains a resin that is capable of performing the unit operation of capturing at a flow rate of between about 0.2 mL/minute to about 100 L/minute (inclusive) (e.g., any of the subranges of this flow rate range described herein). The volume of elution buffer used to elute the recombinant therapeutic protein from each of the at least one chromatographic column or chromatographic membrane containing a resin that is capable of performing the unit operation of purifying can be, e.g., between about 1X column volume (CV) to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, about 4X CV, or about 3X CV (inclusive); between about 2X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, or about 4X CV (inclusive); between about 3X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 5X CV (inclusive); between about 4X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 6X CV (inclusive); between about 5X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, or about 7X CV (inclusive); between about 6X CV

to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV,
 about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about
 12X CV, about 11X CV, about 10X CV, about 9X CV, or about 8X CV (inclusive);
 between about 7X CV to about 30X CV, about 28X CV, about 26X CV, about 24X
 5 CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV,
 about 13X CV, about 12X CV, about 11X CV, about 10X CV, or about 9X CV
 (inclusive); between about 8X CV to about 30X CV, about 28X CV, about 26X CV,
 about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about
 14X CV, about 13X CV, about 12X CV, about 11X CV, or about 10X CV (inclusive);
 10 between about 9 CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV,
 about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about
 13X CV, about 12X CV, or about 11X CV (inclusive); between about 10X CV to
 about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about
 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, or about 12X
 15 CV (inclusive); between about 11X CV to about 30X CV, about 28X CV, about 26X
 CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV,
 about 14X CV, or about 13X CV (inclusive); between about 12X CV to about 30X
 CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV,
 about 18X CV, about 16X CV, or about 14X CV (inclusive); between about 14X CV
 20 to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV,
 about 20X CV, about 18X CV, or about 16X CV (inclusive); between about 16X CV
 to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV,
 about 20X CV, or about 18X CV (inclusive); between about 18X CV to about 30X
 CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, or about 20X CV
 25 (inclusive); between about 20X CV to about 30X CV, about 28X CV, about 26X CV,
 about 24X CV, or about 22X CV (inclusive); between about 22X CV to about 30X
 CV, about 28X CV, about 26X CV, or about 24X CV (inclusive); between about 24X
 CV to about 30X CV, about 28X CV, or about 26X CV (inclusive); between about
 26X CV to about 30X CV or about 28X CV (inclusive); or between about 28X CV to
 30 about 30X CV (inclusive).

The total time of the eluting can be, e.g., between about 2 minutes to about 24
 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14
 hours, about 12 hours, about 10 hours, about 7.5 hours, about 5 hours, about 2.5

hours, about 2.0 hours, about 1.5 hours, about 1.5 hours, about 1.25 hours, about 1
 hour, about 40 minutes, about 30 minutes, about 20 minutes, about 10 minutes, or
 about 5 minutes, inclusive; between about 5 minutes to about 24 hours, about 22
 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12
 5 hours, about 10 hours, about 7.5 hours, about 5 hours, about 2.5 hours, about 2.0
 hours, about 1.5 hours, about 1.5 hours, about 1.25 hours, about 1 hour, about 40
 minutes, about 30 minutes, about 20 minutes, or about 10 minutes, inclusive; between
 about 10 minutes to about 24 hours, about 22 hours, about 20 hours, about 18 hours,
 about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 7.5 hours,
 10 about 5 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.5 hours,
 about 1.25 hours, about 1 hour, about 40 minutes, about 30 minutes, or about 20
 minutes, inclusive; between about 20 minutes to about 24 hours, about 22 hours,
 about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about
 10 hours, about 7.5 hours, about 5 hours, about 2.5 hours, about 2.0 hours, about 1.5
 15 hours, about 1.5 hours, about 1.25 hours, about 1 hour, about 40 minutes, or about 30
 minutes, inclusive; between about 30 minutes to about 24 hours, about 22 hours,
 about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about
 10 hours, about 7.5 hours, about 5 hours, about 2.5 hours, about 2.0 hours, about 1.5
 hours, about 1.5 hours, about 1.25 hours, about 1 hour, or about 40 minutes, inclusive;
 20 between about 40 minutes to about 24 hours, about 22 hours, about 20 hours, about 18
 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 7.5
 hours, about 5 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.5
 hours, about 1.25 hours, or about 1 hour, inclusive; between about 1 hour to about 24
 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14
 25 hours, about 12 hours, about 10 hours, about 7.5 hours, about 5 hours, about 2.5
 hours, about 2.0 hours, about 1.5 hours, about 1.5 hours, or about 1.25 hours,
 inclusive; between about 1.25 hours to about 24 hours, about 22 hours, about 20
 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10
 hours, about 7.5 hours, about 5 hours, about 2.5 hours, about 2.0 hours, about 1.5
 30 hours, or about 1.5 hours, inclusive; between about 1.5 hour to about 24 hours, about
 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12
 hours, about 10 hours, about 7.5 hours, about 5 hours, about 2.5 hours, about 2.0
 hours, or about 1.5 hours, inclusive; between about 1.5 hours to about 24 hours, about

22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 7.5 hours, about 5 hours, about 2.5 hours, or about 2.0 hours, inclusive; between about 2.0 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 7.5 hours, about 5 hours, or about 2.5 hours, inclusive; between about 2.5 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, about 7.5 hours, or about 5 hours, inclusive; between about 5 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, about 10 hours, or about 7.5 hours, inclusive; between about 7.5 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, about 12 hours, or about 10 hours, inclusive; between about 10 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, about 14 hours, or about 12 hours, inclusive; between about 12 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, about 16 hours, or about 14 hours, inclusive; between about 14 hours to about 24 hours, about 22 hours, about 20 hours, about 18 hours, or about 16 hours, inclusive; between about 16 hours to about 24 hours, about 22 hours, about 20 hours, or about 18 hours, inclusive; between about 18 hours to about 24 hours, about 22 hours, or about 20 hours, inclusive; between about 20 hours to about 24 hours or about 22 hours, inclusive; or between about 22 hours to about 24 hours, inclusive.

Non-limiting examples of elution buffers that can be used in these methods will depend on the capture mechanism and/or the therapeutic protein. For example, an elution buffer can contain a different concentration of salt (e.g., increased salt concentration), a different pH (e.g., an increased or decreased salt concentration), or a molecule that will compete with the recombinant therapeutic protein for binding to the resin that is capable of performing the unit operation of capturing. Examples of such elution buffers for each exemplary capture mechanism described herein are well known in the art.

Following the elution of the recombinant therapeutic protein from the at least one chromatographic column or chromatographic membrane in the first MCCS that contains a resin that is capable of performing the unit operation of capturing, and before the next volume of liquid culture medium can be loaded onto the at least one chromatographic column or chromatographic membrane, the at least one

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10X CV, or about 9X CV (inclusive); between about 8X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, or about 10X CV (inclusive); between about 9 CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, or about 12X CV (inclusive); between about 11X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, or about 12X CV (inclusive); between about 14X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, or about 16X CV (inclusive); between about 16X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, or about 18X CV (inclusive); between about 18X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, or about 20X CV (inclusive); between about 20X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, or about 22X CV (inclusive); between about 22X CV to about 30X CV, about 28X CV, about 26X CV, or about 24X CV (inclusive); between about 24X CV to about 30X CV, about 28X CV, or about 26X CV (inclusive); between about 26X CV to about 30X CV or about 28X CV (inclusive); or between about 28X CV to about 30X CV (inclusive).

In some of the processes described herein, the first MCCS includes a reservoir that holds a fluid containing the recombinant therapeutic protein at low pH (e.g., a pH below 4.6, below 4.4, below 4.2, below 4.0, below 3.8, below 3.6, below 3.4, below 3.2, or below 3.0) for, e.g., about 1 minute to 3.0 hours (e.g., about 1 hour), and inactivates the viruses present in a fluid containing the recombinant therapeutic protein. An example of a reservoir that can be used to perform the unit operation of inactivating viruses is a stir flask (e.g., 500-mL stir flask, e.g., a 500-mL stir flask with a programmed stir plate) that is capable of holding a fluid containing a recombinant therapeutic protein for, e.g., about 1 minute to 1.5 hours, before the fluid

containing the recombinant therapeutic protein is fed into the second MCCS. The reservoir that is used to perform the unit operation of inactivation of viruses can be a 500-mL stir flask with a programmed stir plate (e.g., a stir plate programmed to mix (e.g., periodically mix) the fluid within the reservoir, e.g., every 4 hours). Another

5 example of a reservoir that can be used to perform the unit operation of inactivation of viruses is a plastic bag (e.g., 500-mL plastic bag) that is capable of holding a fluid containing a recombinant therapeutic protein for, e.g., about 1 minute to 1.5 hours, before the fluid containing the recombinant therapeutic protein is fed into the second MCCS. In some examples, the fluid containing the recombinant therapeutic protein

10 can already have a low pH (e.g., a pH below 4.6, below 4.4, below 4.2, below 4.0, below 3.8, below 3.6, below 3.4, below 3.2, or below 3.0) when it is fed into the reservoir that is used to perform the unit operation of viral inactivation. Viral inactivation can also be performed using a tubular reactor (e.g., as described in Klutz et al., *Chemical Engineering Processing: Process Intensification* 102:88-101, 2016b;

15 Klutz et al., *Chemical Engineering Research Design* 95:22-33, 2015) or a coiled flow inverter (see, e.g., Klutz et al., *Chemical Engineering Research Design* 95:22-33, 2015; and Klutz et al., *Chemical Engineering Processing: Process Intensification* 102:88-101, 2016b). As can be appreciated by those skilled in the art, a variety of other means can be used to perform the unit operation of inactivating viruses. For

20 example, UV irradiation (e.g., using UV-C light) of a fluid containing recombinant therapeutic protein can also be used to perform the unit operation of inactivating viruses (see, e.g., Lorenz et al., *Biotechnol. Prog.* 25(2):476-482, 2009; Caillet-Fauquet et al., *J. Virol. Methods* 118(2):131-139, 2004; Gunn et al., U.S. Patent No. 6,586,172, 2003; Kaiser et al., U.S. Patent 7,695,675, 2002; Bae et al., *Kor. J. Microbiol. Biotechnol.* 4:377-382, 2009; Li et al., *Biologicals* 33(2):101-110, 2005; and Wang et al., *Vox Sang.* 86(4):230-238, 2004). Non-limiting examples of reservoirs that can be used to perform the unit operation of inactivation of viruses present in a fluid containing the recombinant therapeutic protein are described herein.

The first MCCS can include a PCCS containing four chromatography

30 columns, where at least three of the four chromatography columns perform the unit operation of capturing the recombinant therapeutic protein from the liquid culture medium (e.g., using a first MCCS that includes any of the at least one chromatography columns that contain a resin that is capable of performing the unit

operation of capturing (e.g., any of those described herein)). In these examples, the fourth-column of the PCC can perform the unit operation of inactivating viruses in a fluid that contains the recombinant therapeutic protein (e.g., any of the exemplary columns described herein that can be used to achieve viral inactivation of a fluid containing the recombinant therapeutic protein).

A fluid containing the recombinant therapeutic protein is continuously eluted from the first MCCS (e.g., the first PCC system), and is continuously fed into the second MCCS. The percent of the recombinant therapeutic protein recovered in the eluate of the first MCCS (e.g., the first PCC system) can be, e.g., at least 70%, at least 72%, at least 74%, at least 76%, at least 78%, at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 92%, at least 94%, at least 96%, or at least 98%). The eluate from the first MCCS (e.g., the first PCC system) can be fed into the second MCCS (e.g., second PCC system) using a variety of means known in the art (e.g., tubing). The eluate of the first MCCS (e.g., first PCC system) can be fed into the second MCCS (e.g., second PCC system) at a flow rate of, e.g., between about 0.2 mL/minute to about 100 L/minute (inclusive) (e.g., any of the subranges of this flow rate range described herein).

Some processes described herein can further include a step of adjusting the ionic concentration and/or pH of the eluate from the first MCCS (e.g., first PCC system) before it is fed into the second MCCS (e.g., second PCC system). As described herein, the ionic concentration and/or pH of the eluate from the first MCCS (e.g., first PCC system) can be adjusted (before it is fed into the second MCCS) by adding a buffer to the eluate (e.g., through the use of an in-line buffer adjustment reservoir). The buffer can be added to the eluate from the first MCCS at a flow rate of, e.g., between about 0.2 mL/minute to about 100 L/minute (inclusive) (e.g., any of the subranges of this flow rate range described herein).

The processes described herein can further include a step of holding or storing (and optionally also refrigerating) the eluate from the first MCCS prior to feeding the eluate from the first MCCS into the second MCCS. As described herein, this holding or storing step can be performed using any of the reservoirs (e.g., back-up tanks) described herein.

The processes described herein can also include a step of filtering the eluate from the first MCCS before the eluate is fed into the second MCCS. Any of the

exemplary filters or methods for filtration described herein can be used to filter the eluate from the first MCCS before the eluate is fed into the second MCCS.

Polishing and Purifying the Recombinant Therapeutic Protein

5 The processes described herein include a step of purifying and polishing the recombinant therapeutic protein using a second MCCS, where the eluate from the MCC2 is a therapeutic protein drug substance. The second MCCS can include at least one (e.g., two, three, or four) chromatography column or chromatographic membrane that can be used to perform the unit operation of purifying a recombinant therapeutic
10 protein, and at least one (e.g., two, three, or four) chromatography column or chromatographic membrane that can be used to perform the unit operation of polishing the recombinant therapeutic protein.

 The at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of purifying the recombinant therapeutic
15 protein can contain a resin that utilizes a capture mechanism (e.g., any of the capture mechanisms described herein or known in the art), or a resin that can be used to perform anion exchange, cation exchange, or molecular sieve chromatography. The at least one chromatography column or chromatographic membrane that can be used to perform the unit of operation of polishing the recombinant therapeutic protein can
20 contain a resin can be used to perform anion exchange, cation exchange, or molecular sieve chromatography (e.g., any of the exemplary resins for performing anion exchange, cation exchange, or molecular sieve chromatography described herein or known in the art).

 The size, shape, and volume of the at least one chromatography column or
25 chromatography membrane that can be used to perform the unit of operation of purifying the recombinant therapeutic protein, and/or the size and shape of the at least one chromatographic membrane that can be used to perform the unit of operation of polishing the recombinant membrane can any of combination of the exemplary sizes, shapes, and volumes of chromatography columns or chromatographic membranes
30 described herein. As can be appreciated by one skilled in the art, the step of purifying or polishing a recombinant therapeutic protein can, e.g., include the steps of loading, washing, eluting, and equilibrating the at least one chromatography column or chromatographic membrane used to perform the unit of operation of purifying or

polishing the recombinant therapeutic protein. Typically, the elution buffer coming out of a chromatography column or chromatographic membrane used to perform the unit operation of purifying contains the recombinant therapeutic protein. Typically, the loading and/or wash buffer coming out of a chromatography column or chromatographic membrane used to perform the unit operation of polishing contains the recombinant therapeutic protein.

For example, the size of the at least one chromatography column or chromatographic membrane that can be used to perform unit operation of purifying the recombinant therapeutic protein can have a volume of, e.g., between about 2.0 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 250 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 95 mL, about 90 mL, about 85 mL, about 80 mL, about 75 mL, about 70 mL, about 65 mL, about 60 mL, about 55 mL, about 50 mL, about 45 mL, about 40 mL, about 35 mL, about 30 mL, about 25 mL, about 20 mL, about 15 mL, about 10 mL, or about 5.0 mL (inclusive); between about 5.0 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400 mL, about 350 mL, about 300 mL, about 250 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 95 mL, about 90 mL, about 85 mL, about 80 mL, about 75 mL, about 70 mL, about 65 mL, about 60 mL, about 55 mL, about 50 mL, about 45 mL, about 40 mL, about 35 mL, about 30 mL, about 25 mL, about 20 mL, about 15 mL, or about 10 mL (inclusive); between about 10 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65

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 15 about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L,
 about 70 L, about 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L,
 about 35 L, about 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L,
 about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL, about 750
 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL, about 500 mL,
 20 about 450 mL, about 400 mL, about 350 mL, or about 300 mL (inclusive); between
 about 300 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about
 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about
 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950
 25 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL,
 about 650 mL, about 600 mL, about 550 mL, about 500 mL, about 450 mL, about 400
 mL, or about 350 mL (inclusive); between about 350 mL to 200 L, about 180 L, about
 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L,
 about 80 L, about 75 L, about 70 L, about 65 L, about 60 L, about 55 L, about 50 L,
 30 about 45 L, about 40 L, about 35 L, about 30 L, about 25 L, about 20 L, about 15 L,
 about 10 L, about 5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, about
 800 mL, about 750 mL, about 700 mL, about 650 mL, about 600 mL, about 550 mL,
 about 500 mL, about 450 mL, or about 400 mL (inclusive); between about 400 mL to

200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L,
 about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L,
 about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L,
 about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950 mL, about 900
 5 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL,
 about 600 mL, about 550 mL, about 500 mL, or about 450 mL (inclusive); between
 about 450 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about
 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about
 10 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950
 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL,
 about 650 mL, about 600 mL, about 550 mL, or about 500 mL (inclusive); between
 about 500 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about
 15 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about
 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950
 mL, about 900 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL,
 about 650 mL, about 600 mL, or about 550 mL (inclusive); between about 550 mL to
 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L,
 20 about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L,
 about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L,
 about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950 mL, about 900
 mL, about 850 mL, about 800 mL, about 750 mL, about 700 mL, about 650 mL, or
 about 600 mL (inclusive); between about 600 mL to 200 L, about 180 L, about 160 L,
 25 about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80
 L, about 75 L, about 70 L, about 65 L, about 60 L, about 55 L, about 50 L, about 45
 L, about 40 L, about 35 L, about 30 L, about 25 L, about 20 L, about 15 L, about 10
 L, about 5 L, about 1 L, about 950 mL, about 900 mL, about 850 mL, about 800 mL,
 about 750 mL, about 700 mL, or about 650 mL; between about 650 mL to 200 L,
 30 about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about
 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L, about
 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L, about
 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950 mL, about 900 mL,

about 850 mL, about 800 mL, about 750 mL, or about 700 mL (inclusive); between
 about 700 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about
 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about
 5 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950
 mL, about 900 mL, about 850 mL, about 800 mL, or about 750 mL (inclusive);
 between about 750 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L,
 about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L,
 about 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L,
 10 about 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L,
 about 950 mL, about 900 mL, about 850 mL, or about 800 mL (inclusive); between
 about 800 mL to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about
 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about
 15 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950
 mL, about 900 mL, or about 850 mL (inclusive); between about 850 mL to 200 L,
 about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about
 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L, about
 55 L, about 50 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L, about
 20 20 L, about 15 L, about 10 L, about 5 L, about 1 L, about 950 mL, or about 900 mL
 (inclusive); between about 900 mL to 200 L, about 180 L, about 160 L, about 140 L,
 about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L,
 about 70 L, about 65 L, about 60 L, about 55 L, about 50 L, about 45 L, about 40 L,
 about 35 L, about 30 L, about 25 L, about 20 L, about 15 L, about 10 L, about 5 L,
 25 about 1 L, or about 950 mL (inclusive); between about 950 mL to 200 L, about 180 L,
 about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85
 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L, about 55 L, about 50
 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L, about 20 L, about 15
 L, about 10 L, about 5 L, or about 1 L (inclusive); between about 1 L to 200 L, about
 30 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L,
 about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, about 60 L, about 55 L,
 about 50 L, about 45 L, about 40 L, about 35 L, about 30 L, about 25 L, about 20 L,
 about 15 L, about 10 L, or about 5 L (inclusive); between about 5 L to 200 L, about

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L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, about 65 L, or about 60 L (inclusive); between about 60 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, about 70 L, or about 65 L (inclusive); between about 65 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, about 75 L, or about 70 L (inclusive); between about 70 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, about 80 L, or about 75 L (inclusive); between about 75 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, about 85 L, or about 80 L (inclusive); between about 80 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, about 90 L, or about 85 L (inclusive); between about 85 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 95 L, or about 90 L (inclusive); between about 90 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, or about 95 L (inclusive); between about 95 L to 200 L, about 180 L, about 160 L, about 140 L, about 120 L, or about 100 L (inclusive); between about 100 L to 200 L, about 180 L, about 160 L, about 140 L, or about 120 L (inclusive); between about 120 L to 200 L, about 180 L, about 160 L, or about 140 L (inclusive); between about 140 L to 200 L, about 180 L, or about 160 L (inclusive); between about 160 L to about 200 L or about 180 L (inclusive); or between about 180 L to about 200 L (inclusive).

The flow rate of the fluid containing the recombinant therapeutic protein as it is loaded onto the at least one chromatography column or at least one chromatographic that can be used to perform the unit operation of purifying the recombinant therapeutic protein can be, e.g., between about 0.1 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200 mL/minute, about 100 mL/minute, about 25 mL/minute, about 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute, about 6 mL/minute, about 4 mL/minute, about 3 mL/minute, about 2 mL/minute, about 1 mL/minute, or about 0.5 mL/minute (inclusive); between about 0.5 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5

L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, about 25 mL/minute, about
 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute, about 6 mL/minute,
 about 4 mL/minute, about 3 mL/minute, about 2 mL/minute, or about 1 mL/minute
 5 (inclusive); between about 1 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, about 25 mL/minute, about
 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute, about 6 mL/minute,
 10 about 4 mL/minute, about 3 mL/minute, or about 2 mL/minute (inclusive); between
 about 2 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about
 200 mL/minute, about 100 mL/minute, about 25 mL/minute, about 12.5 mL/minute,
 15 about 10.0 mL/minute, about 8.0 mL/minute, about 6 mL/minute, about 4 mL/minute,
 or about 3 mL/minute (inclusive); between about 3 mL/minute to about 100 L/minute,
 about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, about
 10 L/minute, about 5 L/minute, about 1 L/minute, about 800 mL/minute, about 600
 mL/minute, about 400 mL/minute, about 200 mL/minute, about 100 mL/minute,
 20 about 25 mL/minute, about 12.5 mL/minute, about 10.0 mL/minute, about 8.0
 mL/minute, about 6 mL/minute, or about 4 mL/minute (inclusive); between about 4
 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200
 25 mL/minute, about 100 mL/minute, about 25 mL/minute, about 12.5 mL/minute, about
 10.0 mL/minute, about 8.0 mL/minute, or about 6 mL/minute (inclusive); between
 about 6 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about
 30 200 mL/minute, about 100 mL/minute, about 25 mL/minute, about 12.5 mL/minute,
 about 10.0 mL/minute, or about 8.0 mL/minute (inclusive); between about 8
 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,

about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200
 mL/minute, about 100 mL/minute, about 25 mL/minute, about 12.5 mL/minute, or
 about 10.0 mL/minute (inclusive); between about 10 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 5 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 800
 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200 mL/minute,
 about 100 mL/minute, about 25 mL/minute, or about 12.5 mL/minute (inclusive);
 between about 12.5 mL/minute to about 100 L/minute, about 80 L/minute, about 60
 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 10 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, or about 25 mL/minute
 (inclusive); between about 25 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 15 mL/minute, about 200 mL/minute, or about 100 mL/minute (inclusive); between
 about 100 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, or
 about 200 mL/minute (inclusive); between about 200 mL/minute to about 100
 20 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 800
 mL/minute, about 600 mL/minute, or about 400 mL/minute (inclusive); between
 about 400 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 25 L/minute, about 800 mL/minute, or about 600 mL/minute (inclusive); between about
 600 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about
 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, or about 800 mL/minute (inclusive); between about 800 mL/minute to
 about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about
 30 20 L/minute, about 10 L/minute, about 5 L/minute, or about 1 L/minute (inclusive);
 between about 1 L/minute to about 100 L/minute, about 80 L/minute, about 60
 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, or about 5
 L/minute (inclusive); between about 5 L/minute to about 100 L/minute, about 80

L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, or about 10 L/minute (inclusive); between about 10 L/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, or about 20 L/minute (inclusive); between about 20 L/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, or about 40 L/minute (inclusive); between about 40 L/minute to about 100 L/minute, about 80 L/minute, or about 60 L/minute (inclusive); between about 60 L/minute to 100 L/minute or about 80 L/minute (inclusive); or between about 80 L/minute to about 100 L/minute (inclusive)

The concentration of the recombinant therapeutic protein in the fluid loaded onto the at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of purifying the recombinant therapeutic protein can be, e.g., between about 0.05 mg/mL to about 100 mg/mL recombinant protein (e.g., between about 0.1 mg/mL to about 90 mg/mL, between about 0.1 mg/mL to about 80 mg/mL, between about 0.1 mg/mL to about 70 mg/mL, between about 0.1 mg/mL to about 60 mg/mL, between about 0.1 mg/mL to about 50 mg/mL, between about 0.1 mg/mL to about 40 mg/mL, between about 0.1 mg/mL to about 30 mg/mL, between about 0.1 mg/mL to about 20 mg/mL, between 0.5 mg/mL to about 20 mg/mL, between about 0.1 mg/mL to about 15 mg/mL, between about 0.5 mg/mL to about 15 mg/mL, between about 0.1 mg/mL to about 10 mg/mL, or between about 0.5 mg/mL to about 10 mg/mL recombinant therapeutic protein). The resin in the at least one chromatography column or chromatographic membrane used to perform unit operation of purifying can be a resin that can be used to perform anion exchange or cation exchange chromatography. The resin in the at least one chromatography column or chromatographic membrane that is used to perform the unit operation of purifying can be a cationic exchange resin (e.g., Capto-S resin, GE Healthcare Life Sciences, Piscataway, NJ).

Following the loading of the recombinant therapeutic protein onto the at least one chromatographic column or chromatographic membrane in the second MCCS that can be used to perform the unit operation of purifying the recombinant therapeutic protein, the at least one chromatographic column or chromatographic membrane is washed with at least one washing buffer. As can be appreciated in the art, the at least one (e.g., two, three, or four) washing buffer is meant to elute all proteins that are not the recombinant therapeutic protein from the at least one chromatography column or

chromatographic membrane, while not disturbing the interaction of the recombinant therapeutic protein with the resin or otherwise eluting the recombinant therapeutic protein.

The wash buffer can be passed through the at least one chromatography column or chromatographic membrane at a flow rate of, e.g., between about 0.2 mL/minute to about 100 L/minute (inclusive) (e.g., any of the subranges of this flow rate range described herein). The volume of wash buffer used (e.g., combined total volume of wash buffer used when more than one wash buffer is used) can be, e.g., between about 1X column volume (CV) to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, about 4X CV, or about 3X CV (inclusive); between about 2X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, or about 4X CV (inclusive); between about 3X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 5X CV (inclusive); between about 4X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 6X CV (inclusive); between about 5X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, or about 7X CV (inclusive); between about 6X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, or about 8X CV (inclusive); between about 7X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, or

about 9X CV (inclusive); between about 8X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, or about 10X CV (inclusive); between about 9 CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, or about 11X CV (inclusive); between about 10X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, or about 12X CV (inclusive); between about 11X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, or about 13X CV (inclusive); between about 12X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, or about 14X CV (inclusive); between about 14X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, or about 16X CV (inclusive); between about 16X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, or about 18X CV (inclusive); between about 18X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, or about 20X CV (inclusive); between about 20X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, or about 22X CV (inclusive); between about 22X CV to about 30X CV, about 28X CV, about 26X CV, or about 24X CV (inclusive); between about 24X CV to about 30X CV, about 28X CV, or about 26X CV (inclusive); between about 26X CV to about 30X CV or about 28X CV (inclusive); or between about 28X CV to about 30X CV (inclusive). The total time of the washing can be, e.g., between about 2 minutes to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.25 hours, about 1 hour, about 30 minutes, about 20 minutes, about 10 minutes, or about 5 minutes (inclusive); between about 5 minutes to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.25 hours, about 1 hour, about 30 minutes, about 20 minutes, or about 10 minutes (inclusive); between about

10 minutes to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.25 hours, about 1 hour, about 30 minutes, or about 20 minutes (inclusive); between about
5 20 minutes to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.25 hours, about 1 hour, or about 30 minutes (inclusive); between about 30 minutes to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about
10 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, about 1.25 hours, or about 1 hour (inclusive); between about 1 hour to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, about 2.0 hours, about 1.5 hours, or about 1.25 hours (inclusive); between about 1.25 hours to about 48
15 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, about 2.0 hours, or about 1.5 hours (inclusive); between about 1.5 hours to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28
20 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, about 2.5 hours, or about 2.0 hours (inclusive); between about 2.0 hours to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, about 4 hours, or about 2.5 hours (inclusive); between about 2.5 hours to about 48
25 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, about 8 hours, or about 4 hours (inclusive); between about 4 hours to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, about 12 hours, or about 8 hours (inclusive); between about 8 hours to about 48 hours, about
30 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, about 16 hours, or about 12 hours (inclusive); between about 12 hours to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, about 20 hours, or about 16 hours (inclusive); between about 16 hours to about

48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, about 24 hours, or about 20 hours (inclusive); between about 20 hours to about 48 hours, about 40 hours, about 36 hours, about 32 hours, about 28 hours, or about 24 hours (inclusive); between about 24 hours to about 48 hours, about 40 hours, about 36 hours, about 32 hours, or about 28 hours (inclusive); between about 28 hours to about 48 hours, about 40 hours, about 36 hours, or about 32 hours (inclusive); between about 32 hours to about 48 hours, about 40 hours, or about 36 hours (inclusive); between about 36 hours to about 48 hours or about 40 hours (inclusive); or between about 40 hours to about 48 hours (inclusive).

Following the washing of the at least one chromatographic column or chromatographic membrane in the second MCCS that can be used to perform the unit operation of purifying the recombinant therapeutic protein, the recombinant therapeutic protein is eluted from the at least one chromatographic column or chromatographic membrane by passing an elution buffer through the at least one chromatographic column or chromatographic membrane in the second MCCS that can be used to perform the unit operation of purifying the recombinant therapeutic protein. The elution buffer can be passed through the at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of purifying the recombinant therapeutic protein at a flow rate of, e.g., between about 0.2 mL/minute to about 100 L/minute (inclusive) (e.g., any of the subranges of this flow rate range described herein). The volume of elution buffer used to elute the recombinant therapeutic protein from each the at least one chromatographic column or chromatographic membrane that can be used to perform the unit operation of purifying the recombinant therapeutic protein can be, e.g., between about 1X column volume (CV) to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, about 4X CV, or about 3X CV (inclusive); between about 2X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, or about 4X CV (inclusive); between about 3X CV to about 30X CV, about 28X CV, about 26X CV, about 24X

CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 5X CV (inclusive); between about 4X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 6X CV (inclusive); between about 5X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, or about 7X CV (inclusive); between about 6X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, or about 8X CV (inclusive); between about 7X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, or about 9X CV (inclusive); between about 8X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, or about 10X CV (inclusive); between about 9 CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, or about 11X CV (inclusive); between about 10X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, or about 13X CV (inclusive); between about 12X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, or about 14X CV (inclusive); between about 14X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, or about 16X CV (inclusive); between about 16X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, or about 18X CV (inclusive); between about 18X CV to about 30X

CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, or about 20X CV (inclusive); between about 20X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, or about 22X CV (inclusive); between about 22X CV to about 30X CV, about 28X CV, about 26X CV, or about 24X CV (inclusive); between about 24X CV to about 30X CV, about 28X CV, or about 26X CV (inclusive); between about 26X CV to about 30X CV or about 28X CV (inclusive); or between about 28X CV to about 30X CV (inclusive). The total time of the eluting can be, e.g., between about 2 minutes to about 3 hours (e.g., between about 2 minutes to about 2.5 hours, between about 2 minutes to about 2.0 hours, between about 2 minutes to about 1.5 hours, between about 2 minutes to about 1.5 hours, between about 2 minutes to about 1.25 hours, between about 2 minutes to about 1.25 hours, between about 2 minutes to about 1 hour, between about 2 minutes and about 40 minutes, between about 10 minutes and about 40 minutes, between about 20 minutes and about 40 minutes, or between about 30 minutes and 1.0 hour). Non-limiting examples of elution buffers that can be used in these methods will depend on the resin and/or the therapeutic protein. For example, an elution buffer can contain a different concentration of salt (e.g., increased salt concentration), a different pH (e.g., an increased or decreased salt concentration), or a molecule that will compete with the recombinant therapeutic protein for binding to the resin. Examples of such elution buffers for each of the exemplary capture mechanisms described herein are well known in the art.

Following the elution of the recombinant therapeutic protein from the at least one chromatographic column or chromatographic membrane in the second MCCS that can be used to perform the unit operation of purifying the recombinant therapeutic protein, and before the next volume of fluid containing a recombinant therapeutic protein can be loaded onto the at least one chromatographic column or chromatographic membrane, the at least one chromatography column or chromatographic membrane must be equilibrated using a regeneration buffer. The regeneration buffer can be passed through the at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of purifying the recombinant therapeutic protein at a flow rate of, e.g., between about 0.2 mL/minute to about 100 L/minute (inclusive) (e.g., any of the subranges of this flow rate range described herein). The volume of regeneration buffer used to equilibrate the at least one chromatography column or chromatographic membrane that contains

a resin that can be used to perform the unit operation of purifying the recombinant therapeutic protein can be, e.g., between about 1X column volume (CV) to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, about 4X CV, or about 3X CV (inclusive); between about 2X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, about 6X CV, about 5X CV, or about 4X CV (inclusive); between about 3X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 5X CV (inclusive); between about 4X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, about 7X CV, or about 6X CV (inclusive); between about 5X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, about 8X CV, or about 7X CV (inclusive); between about 6X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, about 9X CV, or about 8X CV (inclusive); between about 7X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, about 10X CV, or about 9X CV (inclusive); between about 8X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, about 11X CV, or about 10X CV (inclusive); between about 9 CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, about 12X CV, or about 11X CV (inclusive); between about 10X CV to about 30X CV, about 28X CV, about 26X

CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, about 13X CV, or about 12X CV (inclusive); between about 11X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, about 14X CV, or about 13X CV (inclusive); between about 12X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, about 16X CV, or about 14X CV (inclusive); between about 14X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, about 18X CV, or about 16X CV (inclusive); between about 16X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, about 20X CV, or about 18X CV (inclusive); between about 18X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, about 22X CV, or about 20X CV (inclusive); between about 20X CV to about 30X CV, about 28X CV, about 26X CV, about 24X CV, or about 22X CV (inclusive); between about 22X CV to about 30X CV, about 28X CV, about 26X CV, or about 24X CV (inclusive); between about 24X CV to about 30X CV, about 28X CV, or about 26X CV (inclusive); between about 26X CV to about 30X CV or about 28X CV (inclusive); or between about 28X CV to about 30X CV (inclusive). The concentration of recombinant therapeutic protein in the eluate of the at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of purifying the recombinant therapeutic protein can be, e.g., between about 0.05 mg/mL to about 100 mg/mL recombinant therapeutic protein (e.g., between about 0.1 mg/mL to about 90 mg/mL, between about 0.1 mg/mL to about 80 mg/mL, between about 0.1 mg/mL to about 70 mg/mL, between about 0.1 mg/mL to about 60 mg/mL, between about 0.1 mg/mL to about 50 mg/mL, between about 0.1 mg/mL to about 40 mg/mL, between about 2.5 mg/mL and about 7.5 mg/mL, between about 0.1 mg/mL to about 30 mg/mL, between about 0.1 mg/mL to about 20 mg/mL, between 0.5 mg/mL to about 20 mg/mL, between about 0.1 mg/mL to about 15 mg/mL, between about 0.5 mg/mL to about 15 mg/mL, between about 0.1 mg/mL to about 10 mg/mL, between about 0.5 mg/mL to about 10 mg/mL, between about 10 mg/mL to about 200 mg/mL, between about 10 mg/mL to about 150 mg/mL, between about 10 mg/mL to about 100 mg/mL, between about 10 mg/mL to about 50 mg/mL, between about 50 mg/mL to about 200 mg/mL, between about 50 mg/mL to about 150 mg/mL, between about 50 mg/mL to about 100 mg/mL, between about 100

mg/mL to about 200 mg/mL, between about 100 mg/mL to about 150 mg/mL, or about 150 mg/mL to about 200 mg/mL recombinant therapeutic protein).

The at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of polishing the recombinant therapeutic protein can contain a resin that can be used to perform cation exchange, anion exchange, or molecular sieve chromatography. As can be appreciated in the art, polishing a recombinant therapeutic protein using the at least one chromatography column or chromatography membrane that can be used to perform the unit operation of polishing the recombinant therapeutic protein in the second MCCS can include, e.g., the steps of loading, chasing, and regenerating the at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of polishing the recombinant therapeutic protein. For example, when the steps of loading, chasing, and regenerating are used to perform the polishing, the recombinant therapeutic protein does not bind the resin in the at least one chromatography column or chromatography membrane in the second MCCS that is used to perform the unit operation of polishing the recombinant therapeutic protein, and the recombinant therapeutic protein is eluted from the at least one chromatography column or chromatographic membrane in the loading and chasing steps, and the regenerating step is used to remove any impurities from the at least one chromatography column or chromatographic membrane before additional fluid containing the recombinant therapeutic protein can be loaded onto the at least one chromatography column or chromatographic membrane. Exemplary flow rates and buffer volumes to be used in each of the loading, chasing, and regenerating steps are described below.

The size, shape, and volume of the at least one chromatography column or chromatography membrane that can be used to perform the unit operation of polishing the recombinant therapeutic protein, and/or the size and shape of the at least one chromatographic membrane that can be used to perform the unit operation of polishing the recombinant therapeutic protein can any of combination of the exemplary sizes, shapes, and volumes of chromatography columns or chromatographic membranes described herein. For example, the size of the at least one chromatography column or chromatographic membrane that can be used to perform the unit operation of polishing the recombinant therapeutic protein can have a volume of, e.g., between about 0.5 mL to about 200 L, about 180 L, about 160 L,

about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, about 30 mL, about 20 mL, about 10 mL, about 5 mL, or about 2.5 mL (inclusive); between about 2.5 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, about 30 mL, about 20 mL, about 10 mL, or about 5 mL (inclusive); between about 5 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, about 30 mL, about 20 mL, or about 10 mL (inclusive); between about 10 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, about 30 mL, or about 20 mL (inclusive); between about 20 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, about 40 mL, or about 30 mL (inclusive); between about 30 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, about 60 mL, or about 40 mL (inclusive); between about 40 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160 mL, about 140 mL, about 120 mL, about 100 mL, about 80 mL, or about 60 mL (inclusive); between about 60 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L,

about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L,
 about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160
 mL, about 140 mL, about 120 mL, about 100 mL, or about 80 mL (inclusive);
 between about 80 mL to about 200 L, about 180 L, about 160 L, about 140 L, about
 5 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about
 1 L, about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL,
 about 160 mL, about 140 mL, about 120 mL, or about 100 mL (inclusive); between
 about 100 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L,
 about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L,
 10 about 800 mL, about 600 mL, about 400 mL, about 200 mL, about 180 mL, about 160
 mL, or about 140 mL (inclusive); between about 140 mL to about 200 L, about 180 L,
 about 160 L, about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40
 L, about 20 L, about 10 L, about 1 L, about 800 mL, about 600 mL, about 400 mL,
 about 200 mL, about 180 mL, or about 160 mL (inclusive); between about 160 mL to
 15 about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about
 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, about
 600 mL, about 400 mL, about 200 mL, or about 180 mL (inclusive); between about
 180 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about
 20 800 mL, about 600 mL, about 400 mL, or about 200 mL (inclusive); between about
 200 mL to about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about
 100 L, about 80 L, about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about
 800 mL, about 600 mL, or about 400 mL (inclusive); between about 400 mL to about
 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about 80 L,
 25 about 60 L, about 40 L, about 20 L, about 10 L, about 1 L, about 800 mL, or about
 600 mL (inclusive); between about 600 mL to about 200 L, about 180 L, about 160 L,
 about 140 L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20
 L, about 10 L, about 1 L, or about 800 mL (inclusive); between about 800 mL to
 about 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, about
 30 80 L, about 60 L, about 40 L, about 20 L, about 10 L, or about 1 L (inclusive);
 between about 1 L to about 200 L, about 180 L, about 160 L, about 140 L, about 120
 L, about 100 L, about 80 L, about 60 L, about 40 L, about 20 L, or about 10 L
 (inclusive); between about 10 L to about 200 L, about 180 L, about 160 L, about 140

L, about 120 L, about 100 L, about 80 L, about 60 L, about 40 L, or about 20 L
 (inclusive); between about 20 L to about 200 L, about 180 L, about 160 L, about 140
 L, about 120 L, about 100 L, about 80 L, about 60 L, or about 40 L (inclusive);
 between about 40 L to about 200 L, about 180 L, about 160 L, about 140 L, about 120
 5 L, about 100 L, about 80 L, or about 60 L (inclusive); between about 60 L to about
 200 L, about 180 L, about 160 L, about 140 L, about 120 L, about 100 L, or about 80
 L (inclusive); between about 80 L to about 200 L, about 180 L, about 160 L, about
 140 L, about 120 L, or about 100 L (inclusive); between about 100 L to about 200 L,
 about 180 L, about 160 L, about 140 L, or about 120 L (inclusive); between about 120
 10 L to about 200 L, about 180 L, about 160 L, or about 140 L (inclusive); between
 about 140 L to about 200 L, about 180 L, or about 160 L (inclusive); between about
 160 L to about 200 L or about 180 L (inclusive); or between about 180 L to about 200
 L (inclusive). The flow rate of the fluid containing the recombinant therapeutic
 protein as it is loaded onto the at least one chromatography column or
 15 chromatographic membrane that can be used to perform the unit operation of
 polishing the recombinant therapeutic protein can be, e.g., between about 0.1
 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200
 20 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, about
 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute, about 6 mL/minute,
 about 4 mL/minute, about 3 mL/minute, about 2 mL/minute, about 1 mL/minute,
 about 0.5 mL/minute, or about 0.2 mL/minute (inclusive); between about 0.2
 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40
 25 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute,
 about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200
 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, about
 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute, about 6 mL/minute,
 about 4 mL/minute, about 3 mL/minute, about 2 mL/minute, or about 1 mL/minute
 30 (inclusive); between about 1 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, about 50 mL/minute, about

25 mL/minute, about 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute,
 about 6 mL/minute, about 4 mL/minute, about 3 mL/minute, or about 2 mL/minute
 (inclusive); between about 2 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 5 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, about 50 mL/minute, about
 25 mL/minute, about 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute,
 about 6 mL/minute, about 4 mL/minute, or about 3 mL/minute (inclusive); between
 about 3 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 10 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about
 200 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute,
 about 12.5 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute, about 6
 mL/minute, or about 4 mL/minute (inclusive); between about 4 mL/minute to about
 15 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 800
 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200 mL/minute,
 about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, about 12.5
 mL/minute, about 10.0 mL/minute, about 8.0 mL/minute, or about 6 mL/minute
 20 (inclusive); between about 6 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, about 50 mL/minute, about
 25 mL/minute, about 12.5 mL/minute, about 10.0 mL/minute, or about 8.0 mL/minute
 25 (inclusive); between about 8 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, about 50 mL/minute, about
 25 mL/minute, about 12.5 mL/minute, or about 10.0 mL/minute (inclusive); between
 30 about 10 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, about
 200 mL/minute, about 100 mL/minute, about 50 mL/minute, about 25 mL/minute, or

about 12.5 mL/minute (inclusive); between about 12.5 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 800
 mL/minute, about 600 mL/minute, about 400 mL/minute, about 200 mL/minute,
 5 about 100 mL/minute, about 50 mL/minute, or about 25 mL/minute (inclusive);
 between about 25 mL/minute to about 100 L/minute, about 80 L/minute, about 60
 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, about 100 mL/minute, or about 50 mL/minute
 10 (inclusive); between about 50 mL/minute to about 100 L/minute, about 80 L/minute,
 about 60 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5
 L/minute, about 1 L/minute, about 800 mL/minute, about 600 mL/minute, about 400
 mL/minute, about 200 mL/minute, or about 100 mL/minute (inclusive); between
 about 100 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 15 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 800 mL/minute, about 600 mL/minute, about 400 mL/minute, or
 about 200 mL/minute (inclusive); between about 200 mL/minute to about 100
 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about 20
 L/minute, about 10 L/minute, about 5 L/minute, about 1 L/minute, about 800
 20 mL/minute, about 600 mL/minute, or about 400 mL/minute (inclusive); between
 about 400 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute,
 about 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, about 800 mL/minute, or about 600 mL/minute (inclusive); between about
 600 mL/minute to about 100 L/minute, about 80 L/minute, about 60 L/minute, about
 25 40 L/minute, about 20 L/minute, about 10 L/minute, about 5 L/minute, about 1
 L/minute, or about 800 mL/minute (inclusive); between about 800 mL/minute to
 about 100 L/minute, about 80 L/minute, about 60 L/minute, about 40 L/minute, about
 20 L/minute, about 10 L/minute, about 5 L/minute, or about 1 L/minute (inclusive);
 between about 1 L/minute to about 100 L/minute, about 80 L/minute, about 60
 30 L/minute, about 40 L/minute, about 20 L/minute, about 10 L/minute, or about 5
 L/minute (inclusive); between about 5 L/minute to about 100 L/minute, about 80
 L/minute, about 60 L/minute, about 40 L/minute, about 20 L/minute, or about 10
 L/minute (inclusive); between about 10 L/minute to about 100 L/minute, about 80

L/minute, about 60 L/minute, about 40 L/minute, or about 20 L/minute (inclusive);
 between about 20 L/minute to about 100 L/minute, about 80 L/minute, about 60
 L/minute, or about 40 L/minute (inclusive); between about 40 L/minute to about 100
 L/minute, about 80 L/minute, or about 60 L/minute (inclusive); between about 60
 5 L/minute to about 100 L/minute or about 80 L/minute (inclusive); or between about
 80 L/minute to about 100 L/minute (inclusive). The total volume of fluid containing a
 recombinant therapeutic protein loaded onto the at least one chromatography column
 or chromatographic membrane that can be used to perform the unit operation of
 polishing the recombinant therapeutic protein can be, e.g., between about 1.0 mL to
 10 about 1,000L, about 900 L, about 800 L, about 700 L, about 600 L, about 500 L,
 about 400 L, about 300 L, about 200 L, about 100 L, about 50 L, about 25 L, about 10
 L, about 5 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 250 mL,
 about 225 mL, about 200 mL, about 175 mL, about 150 mL, about 125 mL, about 100
 mL, about 75 mL, about 50 mL, about 25 mL, about 10 mL, or about 5 mL
 15 (inclusive); between about 5 mL to about 1,000L, about 900 L, about 800 L, about
 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about 100 L,
 about 50 L, about 25 L, about 10 L, about 5 L, about 1 L, about 800 mL, about 600
 mL, about 400 mL, about 250 mL, about 225 mL, about 200 mL, about 175 mL,
 about 150 mL, about 125 mL, about 100 mL, about 75 mL, about 50 mL, about 25
 20 mL, or about 10 mL (inclusive); between about 10 mL to about 1,000L, about 900 L,
 about 800 L, about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about
 200 L, about 100 L, about 50 L, about 25 L, about 10 L, about 5 L, about 1 L, about
 800 mL, about 600 mL, about 400 mL, about 250 mL, about 225 mL, about 200 mL,
 about 175 mL, about 150 mL, about 125 mL, about 100 mL, about 75 mL, about 50
 25 mL, or about 25 mL (inclusive); between about 25 mL to about 1,000L, about 900 L,
 about 800 L, about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about
 200 L, about 100 L, about 50 L, about 25 L, about 10 L, about 5 L, about 1 L, about
 800 mL, about 600 mL, about 400 mL, about 250 mL, about 225 mL, about 200 mL,
 about 175 mL, about 150 mL, about 125 mL, about 100 mL, about 75 mL, or about 50
 30 mL (inclusive); between about 50 mL to about 1,000L, about 900 L, about 800 L,
 about 700 L, about 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about
 100 L, about 50 L, about 25 L, about 10 L, about 5 L, about 1 L, about 800 mL, about
 600 mL, about 400 mL, about 250 mL, about 225 mL, about 200 mL, about 175 mL,

about 150 mL, about 125 mL, about 100 mL, or about 75 mL (inclusive); between
 about 75 mL to about 1,000L, about 900 L, about 800 L, about 700 L, about 600 L,
 about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 50 L, about
 25 L, about 10 L, about 5 L, about 1 L, about 800 mL, about 600 mL, about 400 mL,
 5 about 250 mL, about 225 mL, about 200 mL, about 175 mL, about 150 mL, about 125
 mL, or about 100 mL (inclusive); between about 100 mL to about 1,000L, about 900
 L, about 800 L, about 700 L, about 600 L, about 500 L, about 400 L, about 300 L,
 about 200 L, about 100 L, about 50 L, about 25 L, about 10 L, about 5 L, about 1 L,
 about 800 mL, about 600 mL, about 400 mL, about 250 mL, about 225 mL, about 200
 10 mL, about 175 mL, about 150 mL, or about 125 mL (inclusive); between about 125
 mL to about 1,000L, about 900 L, about 800 L, about 700 L, about 600 L, about 500
 L, about 400 L, about 300 L, about 200 L, about 100 L, about 50 L, about 25 L, about
 10 L, about 5 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 250
 mL, about 225 mL, about 200 mL, about 175 mL, or about 150 mL (inclusive);
 15 between about 150 mL to about 1,000L, about 900 L, about 800 L, about 700 L, about
 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 50 L,
 about 25 L, about 10 L, about 5 L, about 1 L, about 800 mL, about 600 mL, about 400
 mL, about 250 mL, about 225 mL, about 200 mL, or about 175 mL (inclusive);
 between about 175 mL to about 1,000L, about 900 L, about 800 L, about 700 L, about
 20 600 L, about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 50 L,
 about 25 L, about 10 L, about 5 L, about 1 L, about 800 mL, about 600 mL, about 400
 mL, about 250 mL, about 225 mL, or about 200 mL (inclusive); between about 200
 mL to about 1,000L, about 900 L, about 800 L, about 700 L, about 600 L, about 500
 L, about 400 L, about 300 L, about 200 L, about 100 L, about 50 L, about 25 L, about
 25 10 L, about 5 L, about 1 L, about 800 mL, about 600 mL, about 400 mL, about 250
 mL, or about 225 mL (inclusive); between about 225 mL to about 1,000L, about 900
 L, about 800 L, about 700 L, about 600 L, about 500 L, about 400 L, about 300 L,
 about 200 L, about 100 L, about 50 L, about 25 L, about 10 L, about 5 L, about 1 L,
 about 800 mL, about 600 mL, about 400 mL, or about 250 mL (inclusive); between
 30 about 250 mL to about 1,000L, about 900 L, about 800 L, about 700 L, about 600 L,
 about 500 L, about 400 L, about 300 L, about 200 L, about 100 L, about 50 L, about
 25 L, about 10 L, about 5 L, about 1 L, about 800 mL, about 600 mL, or about 400
 mL (inclusive); between about 400 mL to about 1,000L, about 900 L, about 800 L,

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 277

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JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 277

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

WHAT IS CLAIMED IS:

1. A method of processing a fluid comprising a recombinant therapeutic protein, the method comprising:

(a) providing a circuit system comprising (i) a tangential flow filtration (TFF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets, comprising at least one port for flowing fluid into or out of, or both, of the system,

wherein the system is configured such that fluid can be flowed through the conduit and the TFF unit, and filtrate not comprising the recombinant therapeutic protein can be collected from the TFF unit;

(b) continuously flowing a fluid comprising a recombinant therapeutic protein into the circuit system through one of the at least one port, and discarding filtrate not comprising the recombinant therapeutic protein for a first period of time;

(c) continuously flowing a diafiltration medium into the circuit system through one of the at least one port, and discarding filtrate not comprising the recombinant therapeutic protein for a second period of time; and

(d) collecting a fluid comprising the recombinant therapeutic protein that is present in the circuit system after the first and second time periods through one of the at least one port.

2. The method of claim 1, wherein step (b) comprises continuously flowing the fluid into the circuit system at a rate of about 0.1 mL/minute to about 100 L/minute.

3. The method of claim 2, wherein step (b) comprises continuously flowing the fluid into the circuit system at a rate of about 0.5 mL/minute to about 200 mL/minute.

4. The method of any one of claims 1-3, wherein the first period of time is about 0.1 minute to about 12 hours.

5. The method of claim 4, wherein the first period of time is about 10 minutes to about 4 hours.

6. The method of any one of claims 1-5, wherein the flowing of the fluid into the circuit system in step (b) occurs unidirectionally.

7. The method of any one of claims 1-6, wherein step (c) comprising continuously flowing the diafiltration medium into the circuit system at a rate of about 0.1 mL/minute to about 100 L/minute.

8. The method of claim 7, wherein step (c) comprises continuously flowing the diafiltration medium into the circuit system at a rate of about 0.5 mL/minute to about 200 mL/minute.

9. The method of any one of claims 1-8, wherein the second period of time is about 5 minutes to about 12 hours.

10. The method of claim 9, wherein the second period of time is about 10 minutes to about 4 hours.

11. The method of any one of claims 1-10, wherein the flowing of the diafiltration medium into the circuit system in step (c) occurs unidirectionally.

12. The method of any one of claims 1-11, wherein the conduit has an inner diameter of about 5 mm to about 5 cm.

13. The method of claim 12, wherein the conduit has an inner diameter of about 10 mm to about 3 cm.

14. The method of any one of claims 1-13, wherein the TFF unit comprises one or more tangential filter(s) having a surface area of about 0.1 cm² to about 50 cm².

15. The method of claim 14, wherein the TFF unit comprises one or more tangential filter(s) having a surface area of about 0.5 cm² to about 20 cm².

16. The method of any one of claims 1-15, wherein circuit system further comprises a pump disposed in the conduit.

17. The method of claim 16, wherein the pump is a peristaltic pump.

18. The method of any one of claims 1-17, further comprising:

(e) providing a second circuit system comprising (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets of the TFVF unit, comprising at least one port for flowing fluid into or out of, or both, of the second system,

wherein the second system is configured such that fluid can be flowed through the conduit and the TFVF unit, and filtrate comprising the recombinant therapeutic protein can be collected from the TFVF unit; and

(f) flowing the collected fluid of step (d) into the second system using a connecting conduit in fluid communication between a port in the system and a port in the second system, and collecting filtrate comprising the recombinant therapeutic protein from the TFVF unit for a third period of time.

19. The method of claim 18, wherein step (f) comprising flowing the fluid into the second system at a rate of about 0.1 mL/minute to about 100 L/minute.

20. The method of claim 19, wherein step (f) comprises flowing the fluid into the second system at a rate of about 0.5 mL/minute to about 200 mL/minute.

21. The method of any one of claims 18-20, wherein the third period of time is about 0.1 minute to about 12 hours.

22. The method of claim 21, wherein the third period of time is about 10 minutes to about 2 hours.

23. The method of any one of claims 18-22, wherein the flowing of the fluid into the second system in step (f) occurs unidirectionally.

24. The method of any one of claims 18-23, wherein the conduit in fluid communication between the first and second inlets of the TFVF unit has an inner diameter of about 5 mm to about 5 cm.

25. The method of claim 24, wherein the conduit in fluid communication between the first and second inlets of the TFVF unit has an inner diameter of about 10 mm to about 3 cm.

26. The method of any one of claims 18-25, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.1 cm² to about 100 m².

27. The method of claim 26, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.5 cm² to about 200 cm².

28. The method of any one of claims 18-27, wherein the connecting conduit has an inner diameter of about 5 mm to about 10 cm.

29. The method of claim 28, wherein the connecting conduit has an inner diameter of about 10 mm to about 5 cm.

30. The method of any one of claims 18-29, wherein second system further comprises a pump disposed in the conduit in fluid communication between the first and second inlets of the TFVF unit.

31. The method of claim 30, wherein the pump disposed in the conduit in fluid communication between the first and second inlets of the TFVF unit is a peristaltic pump.

32. A method of processing a fluid comprising a recombinant therapeutic protein, the method comprising:

(a) providing a circuit system comprising (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a conduit in fluid communication

between the first and second inlets of the TFVF unit, comprising at least one port for flowing fluid into or out of, or both, of the system,

wherein the system is configured such that fluid can be flowed through the conduit and the TFVF unit, and filtrate comprising the recombinant therapeutic protein can be collected from the TFVF unit;

(b) continuously flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port, and collecting filtrate comprising the recombinant therapeutic protein from the TFVF unit for a period of time.

33. The method of claim 32, wherein step (b) comprising continuously flowing the fluid into the system at a rate of about 0.1 mL/minute to about 100 L/minute.

34. The method of claim 33, wherein step (b) comprises continuously flowing the fluid into the system at a rate of about 0.5 mL/minute to about 200 mL/minute.

35. The method of any one of claims 32-34, wherein the period of time is about 5 minutes to about 12 hours.

36. The method of claim 35, wherein the period of time is about 10 minutes to about 3 hours.

37. The method of any one of claims 32-36, wherein the flowing of the fluid into the system in step (b) occurs unidirectionally.

38. The method of any one of claims 32-37, wherein the conduit has an inner diameter of about 5 mm to about 5 cm.

39. The method of claim 38, wherein the conduit has an inner diameter of about 10 mm to about 3 cm.

40. The method of any one of claims 32-39, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.1 cm² to about 100 m².

41. The method of claim 40, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.5 cm² to about 200 cm².

42. The method of any one of claims 32-41, wherein system further comprises a pump disposed in the conduit.

43. The method of claim 42, wherein the pump disposed in the conduit is a peristaltic pump.

44. An integrated and continuous process for manufacturing a therapeutic protein drug substance, the process comprising:

(a) providing a liquid culture medium comprising a recombinant therapeutic protein that is substantially free of cells, wherein the liquid culture medium is fed into a first multi-column chromatography system (MCCS1);

(b) capturing said recombinant therapeutic protein in the liquid culture medium using the MCCS1, wherein the eluate of the MCCS1 containing the recombinant therapeutic protein is continuously fed into a second multi-column chromatography system (MCCS2);

(c) purifying and polishing the recombinant therapeutic protein using the MCCS2, wherein the eluate from the MCCS2 is a fluid comprising the recombinant therapeutic protein;

(d) processing the fluid comprising the recombinant therapeutic protein using a method of any one of claims 1-43,

wherein the process is integrated and runs continuously from said liquid culture medium to the collected fluid or filtrate comprising the recombinant therapeutic protein, wherein the collected fluid or filtrate is the therapeutic protein drug substance.

45. The process of claim 44, wherein the MCCS1 performs at least two different unit operations.

46. The process of claim 45, wherein the MCCS1 performs the unit operations of capturing the recombinant therapeutic protein and inactivating viruses.

47. The process of any one of claims 44-46, wherein the use of the MCCS1 or the MCCS2, or both, involves column switching.

48. The process of any one of claims 44-47, wherein the MCCS1 and/or MCCS2 utilizes at least two chromatography columns.

49. The process of any one of claims 44-47, wherein the MCCS1 and/or MCCS2 utilizes at least two chromatographic membranes.

50. The process of any one of claims 44-47, wherein the MCCS1 and/or MCCS2 utilizes at least one chromatography column and at least one chromatographic membrane.

51. The process of any one of claims 44-50, wherein the MCCS1 is a first periodic counter current chromatography system (PCCS1).

52. The process of claim 51, wherein the PCCS1 comprises a four-column PCCS.

53. The process of claim 52, wherein three of the four columns in the four-column PCCS perform the unit operation of capturing the recombinant therapeutic protein from the liquid culture medium.

54. The process of claim 53, wherein the capturing is performed using affinity chromatography, cation exchange chromatography, anion exchange chromatography, or molecular sieve chromatography.

55. The process of claim 54, wherein the affinity chromatography is performed with a capture mechanism selected from the group consisting of: protein A-binding capture mechanism, substrate-binding capture mechanism, antibody- or antibody fragment-binding capture mechanism, aptamer-binding capture mechanism, and cofactor-binding capture mechanism.

56. The process of claim 55, wherein the affinity chromatography is performed with a protein A-binding capture mechanism, and the recombinant therapeutic protein is an antibody or an antibody fragment.

57. The process of any one of claims 53-56, wherein the eluate containing the recombinant therapeutic protein from three of the four columns in the four-column PCCS is fed into the fourth column of the four-column PCCS.

58. The process of claim 57, wherein the fourth column of the four-column PCCS performs the unit operation of inactivating viruses by holding the eluate containing recombinant therapeutic protein at a low pH for viral inactivation.

59. The process of claim 58, wherein the fourth column of the four-column PCCS holds the eluate containing the recombinant protein at a low pH for viral inactivation for a period of about 10 minutes to about 1.5 hours.

60. The process of claim 58, wherein the MCCS2 is a second periodic counter current chromatography system (PCCS2).

61. The process of claim 60, further comprising adjusting the pH of the eluate from the fourth column of the four-column PCCS using an in-line buffer adjustment reservoir before the eluate from the fourth column of the four-column PCCS is fed into the PCCS2.

62. The process of claim 61, wherein the PCCS2 comprises three chromatography columns and a chromatographic membrane.

63. The process of claim 62, wherein the three chromatographic columns in the PCCS2 perform the unit operation of purifying the recombinant therapeutic protein from the eluate of the PCCS1 through cation or anion exchange chromatography.

64. The process of claim 63, wherein the eluate from the three chromatography columns in the PCCS2 is fed into the chromatographic membrane in the PCCS2.

65. The process of claim 64, wherein the chromatographic membrane in the PCCS2 performs the unit operation of polishing the recombinant therapeutic protein present in the eluate from the three chromatographic columns in the PCCS2 through cation or anion exchange chromatography.

66. The process of claim 65, wherein the chromatographic membrane in the PCCS2 performs the unit operation of polishing through cation exchange chromatography.

67. The process of claim 66, wherein the flow through and wash of the chromatographic membrane comprises the recombinant therapeutic protein.

68. The process of claim 57, further comprising adjusting the ionic concentration of the eluate from the three columns in the PCCS2 using in-line buffer adjustment before the eluate from the three columns in the PCCS2 is fed into the chromatographic membrane in the PCCS2.

69. The process of claim 60, further comprising the use of a break tank between the PCCS1 and the PCCS2.

70. The process of claim 60, further comprising filtering the eluate from the PCCS1 before it is fed into the PCCS2.

71. The process of any one of claims 44-70, further comprising formulating the therapeutic protein drug substance into a pharmaceutical composition.

72. The method of any one of claims 44-55 and 57-71, wherein the recombinant therapeutic protein is an antibody or antibody fragment, an enzyme, an engineered protein, or an immunogenic protein or protein fragment.

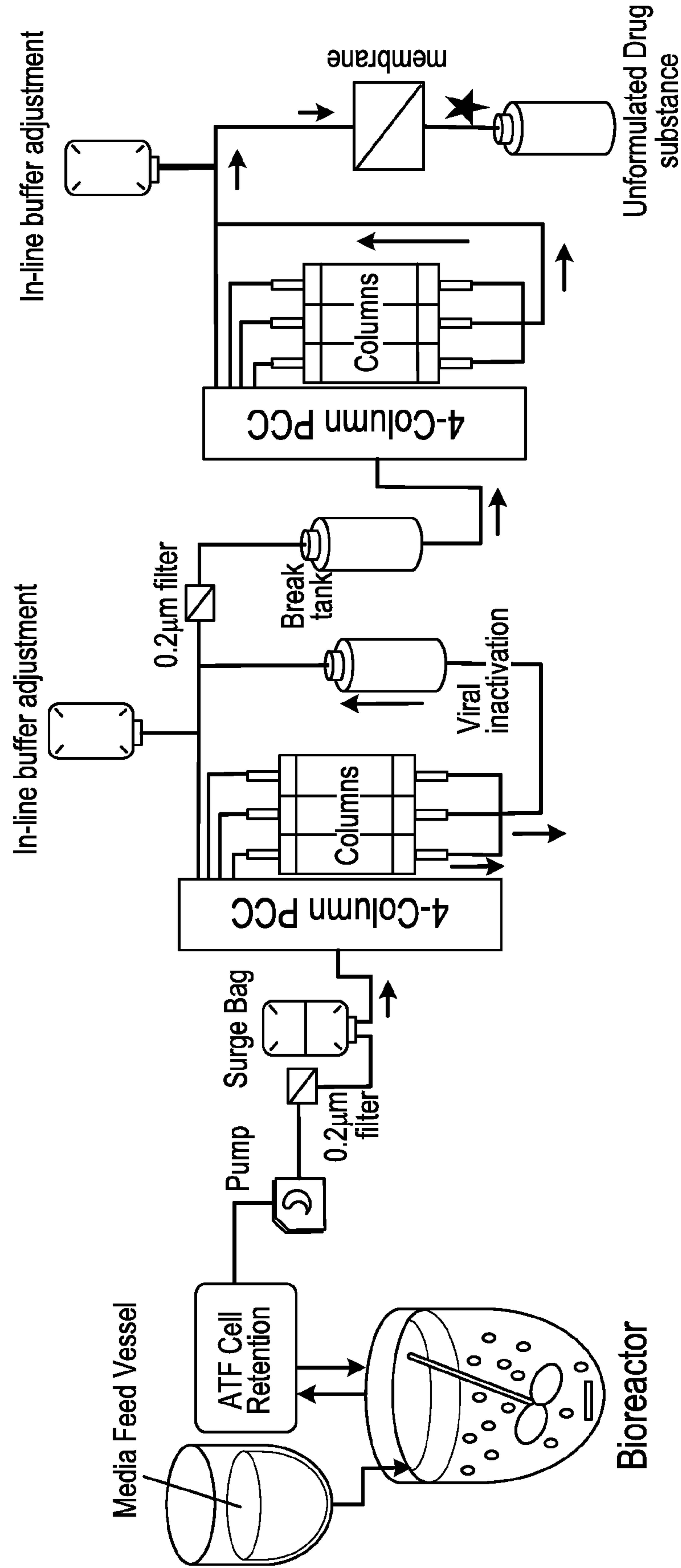


FIG. 1

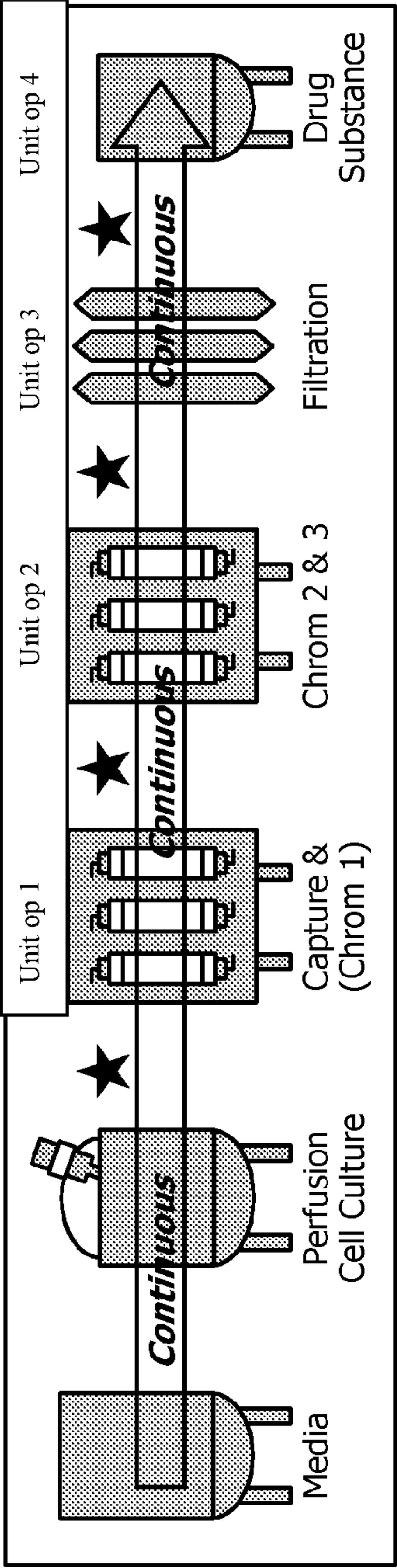


FIG. 2

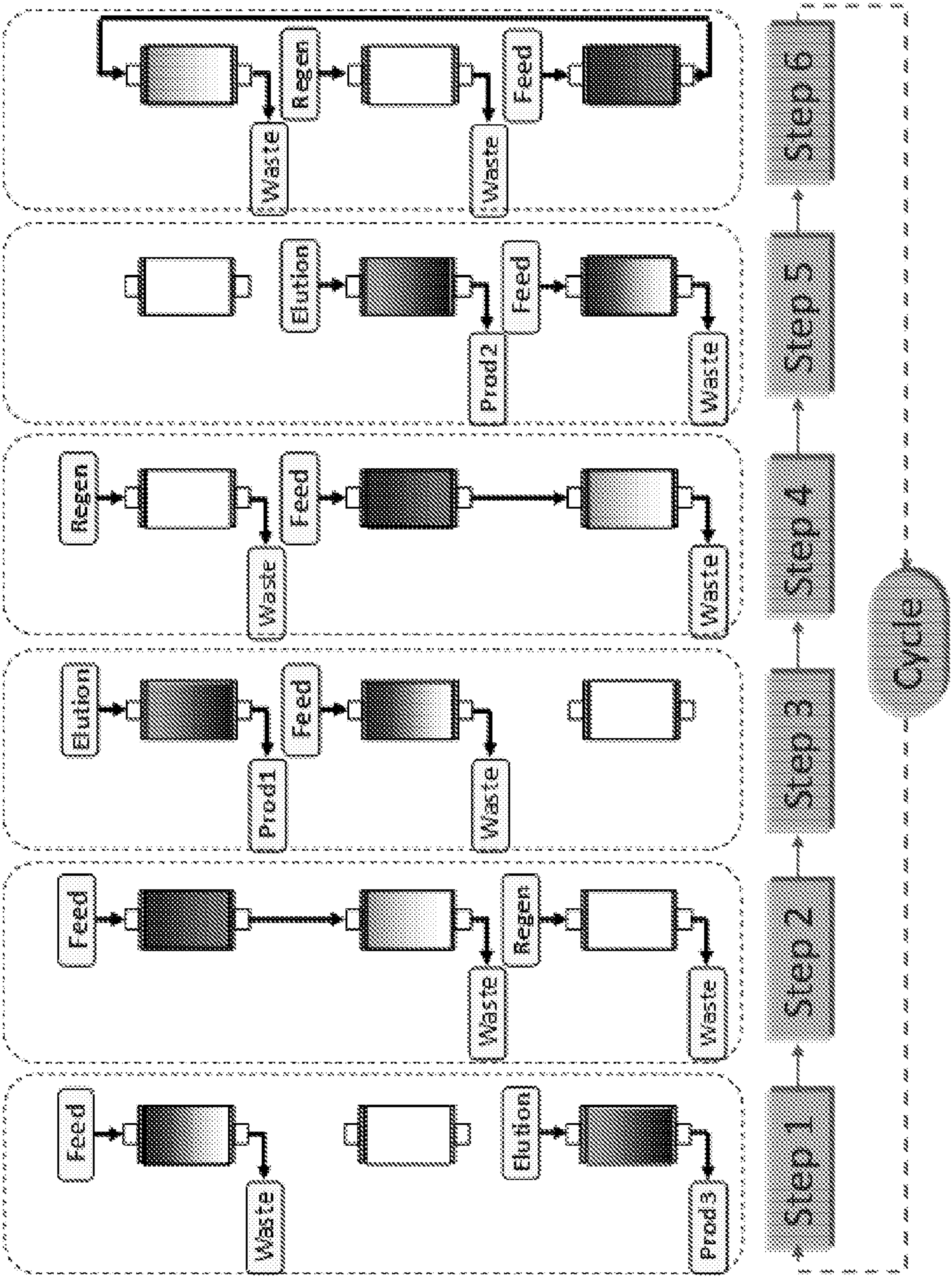


FIG. 3

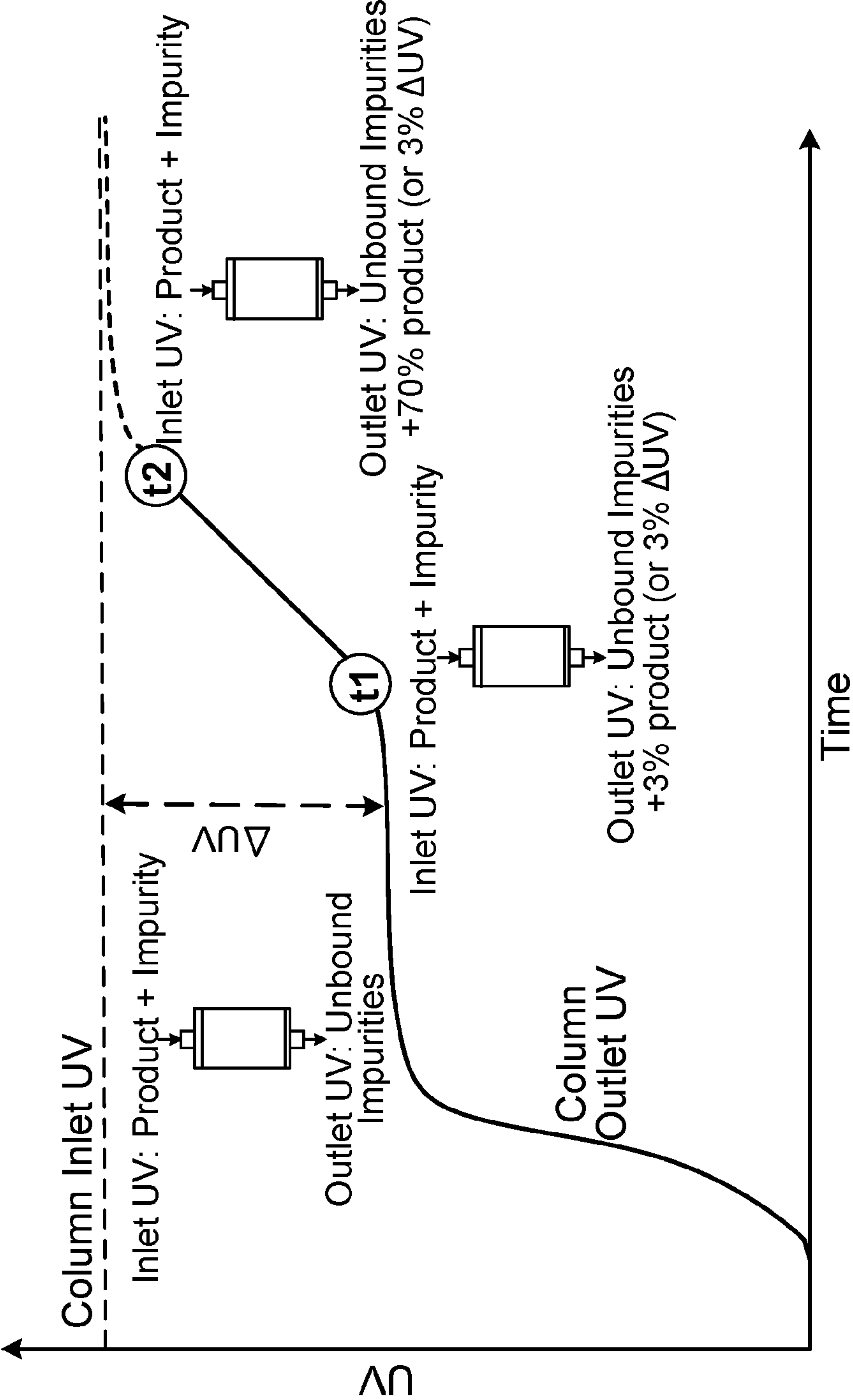


FIG. 4

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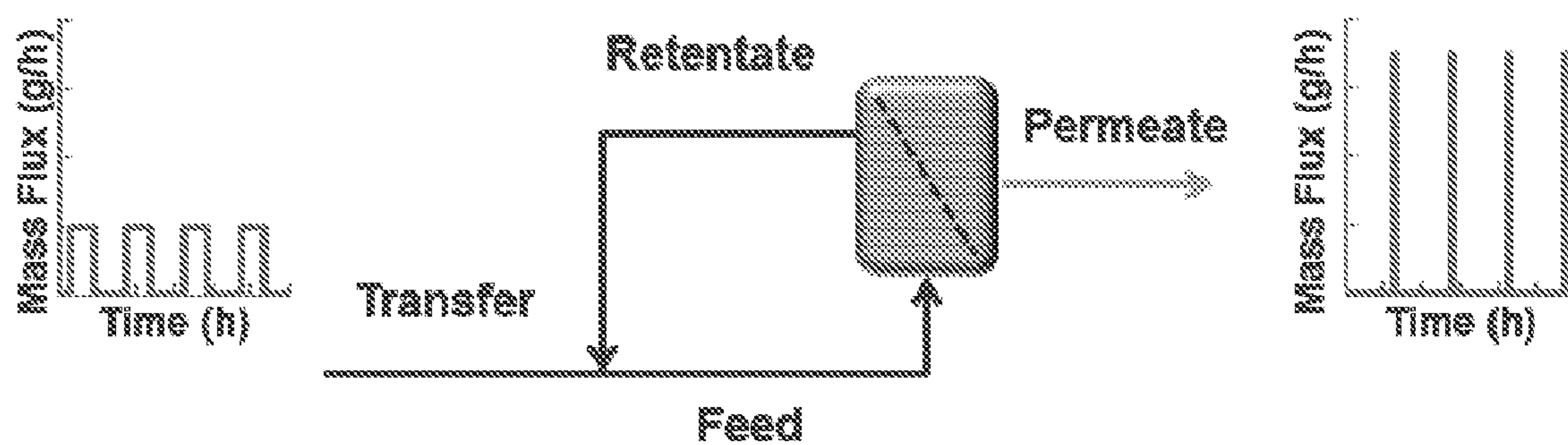


FIG. 5

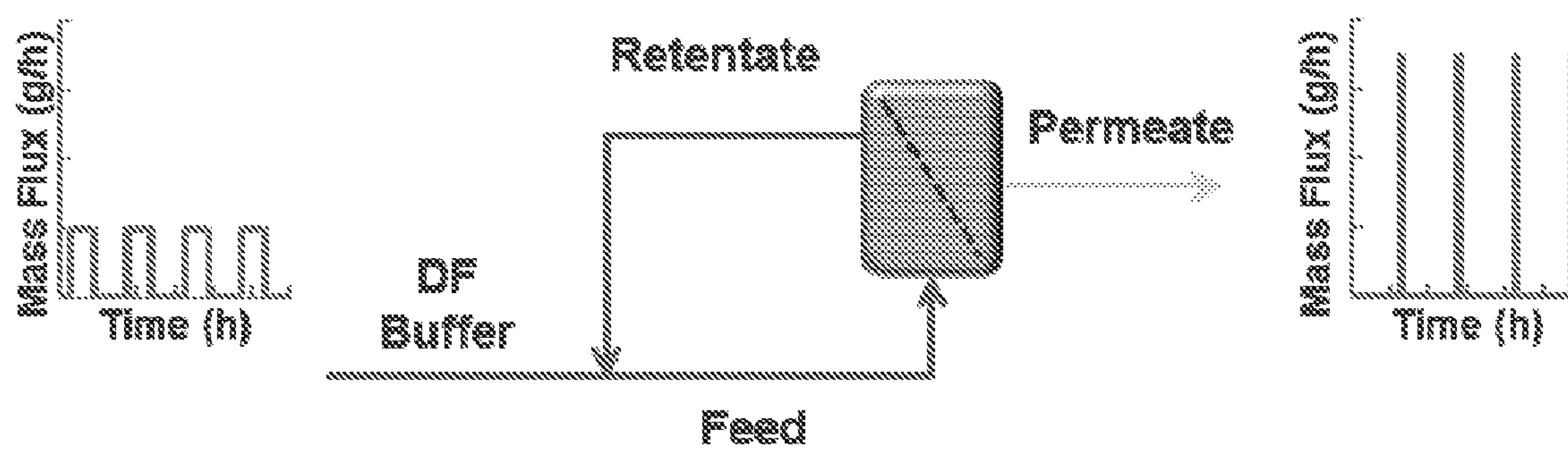


FIG. 6

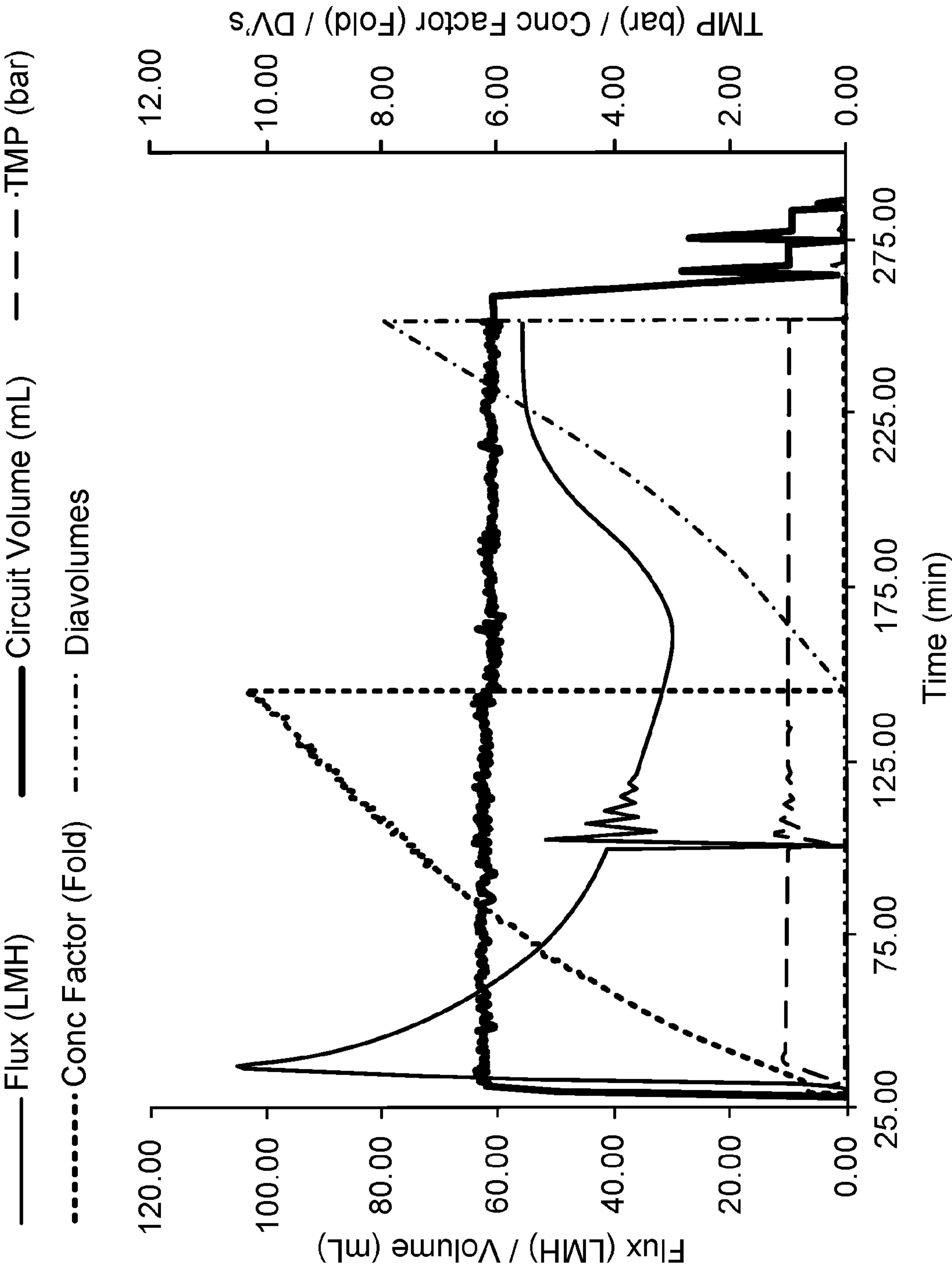


FIG. 7

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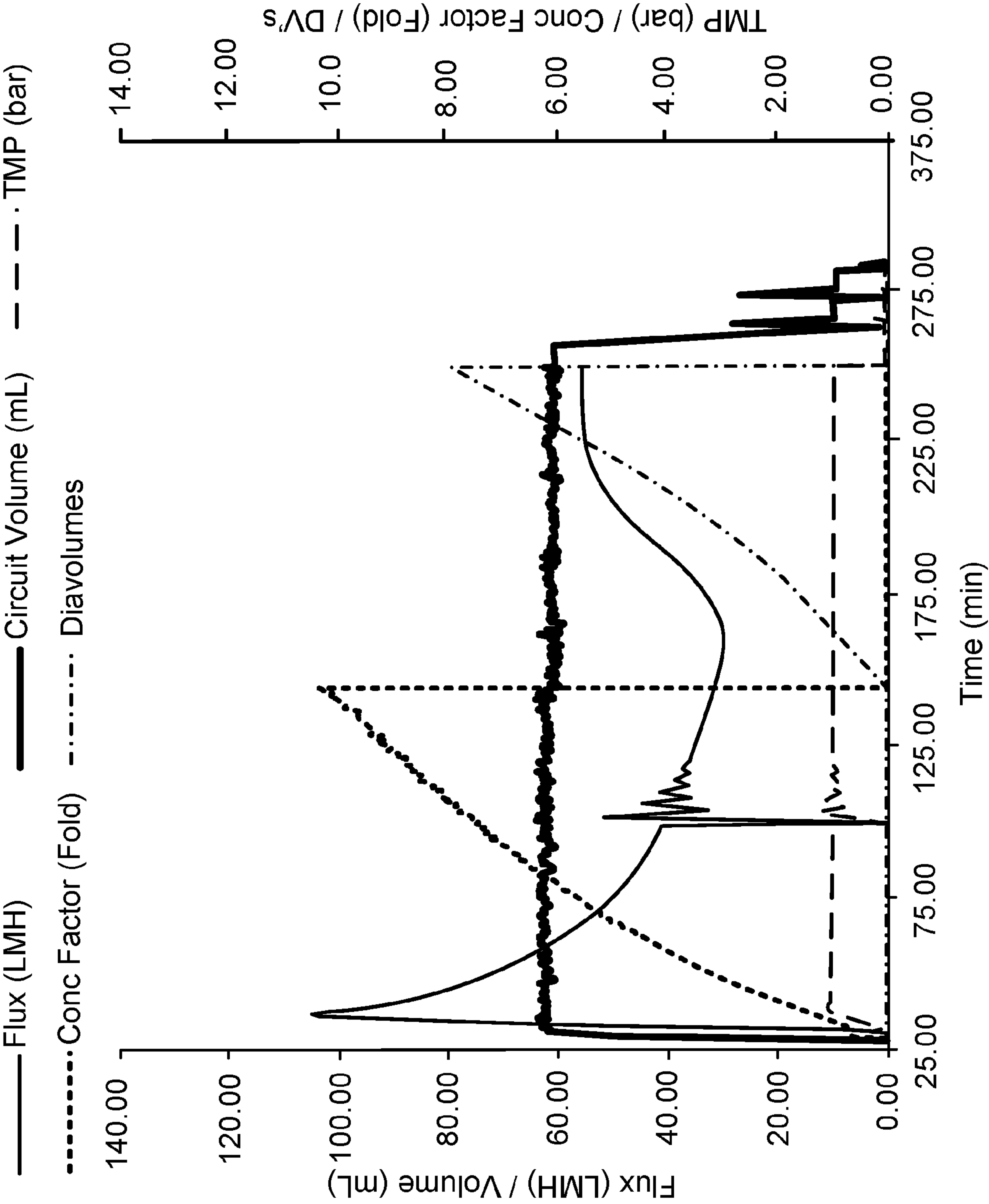


FIG. 8

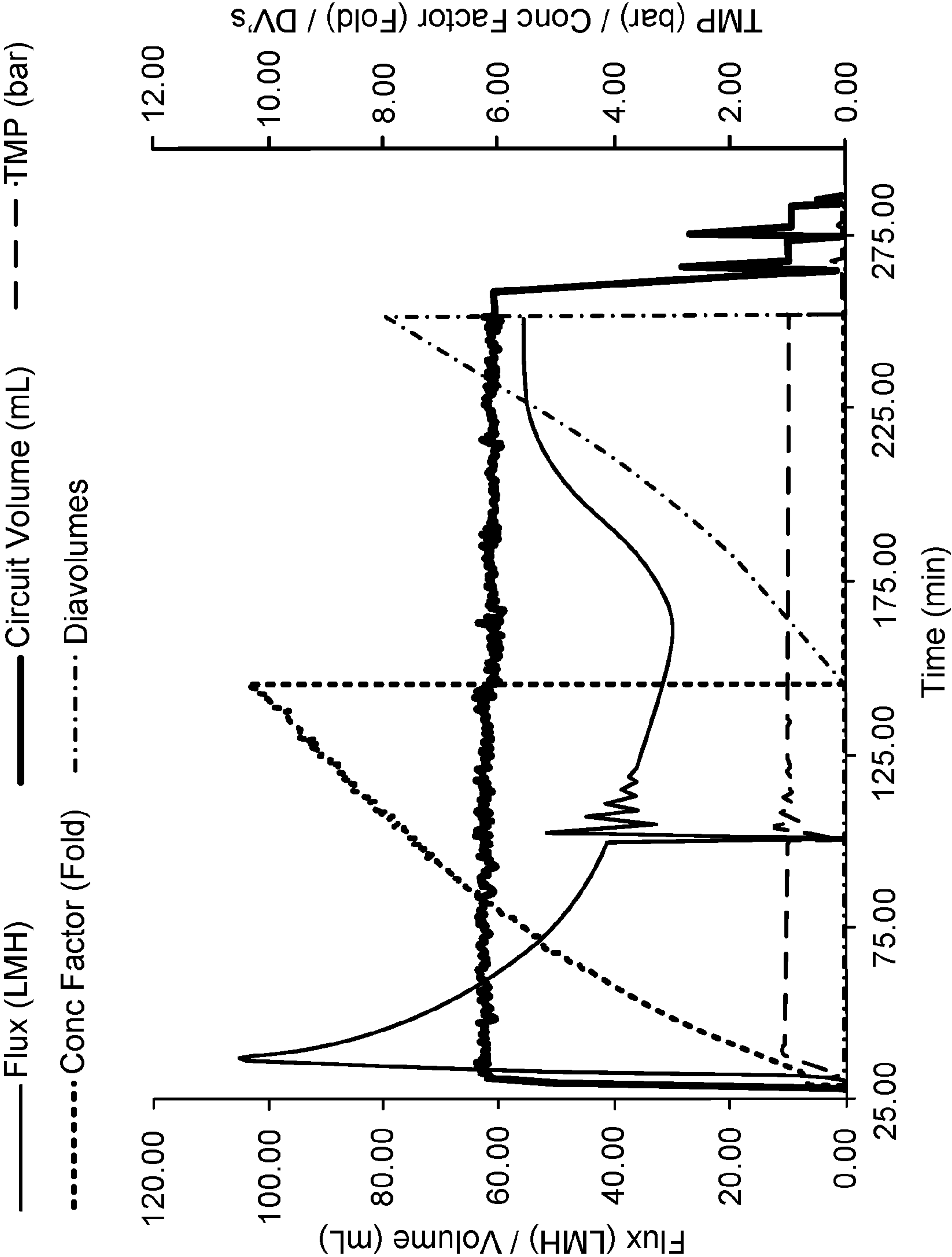


FIG. 9

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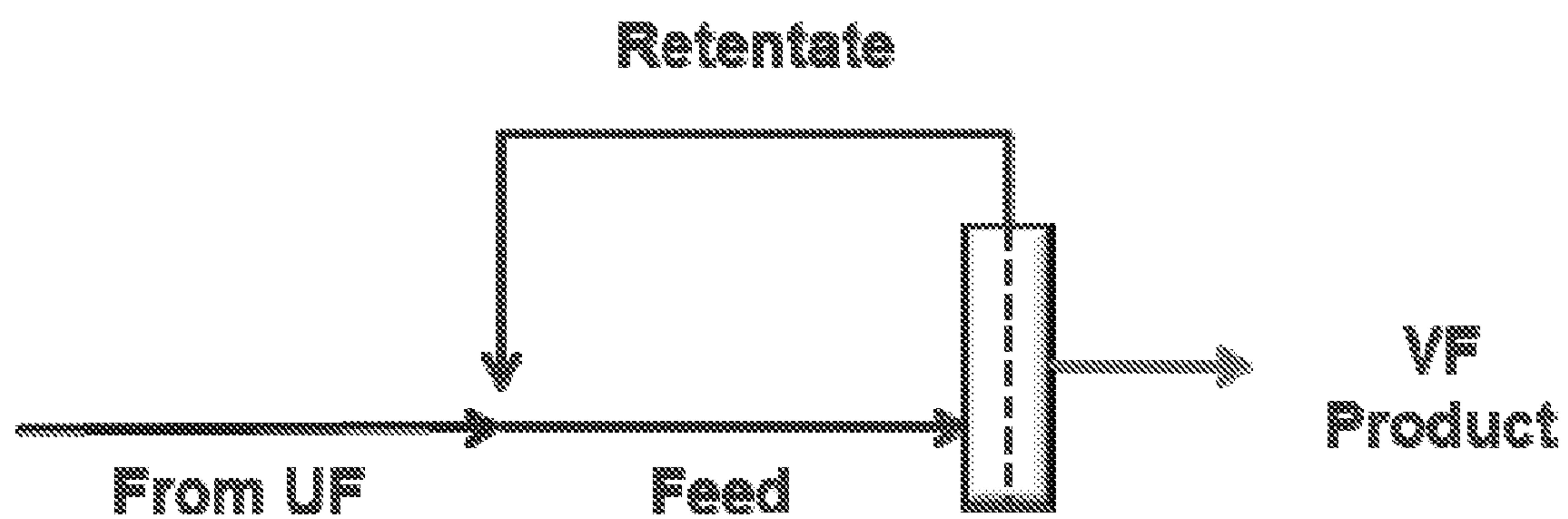


FIG. 10

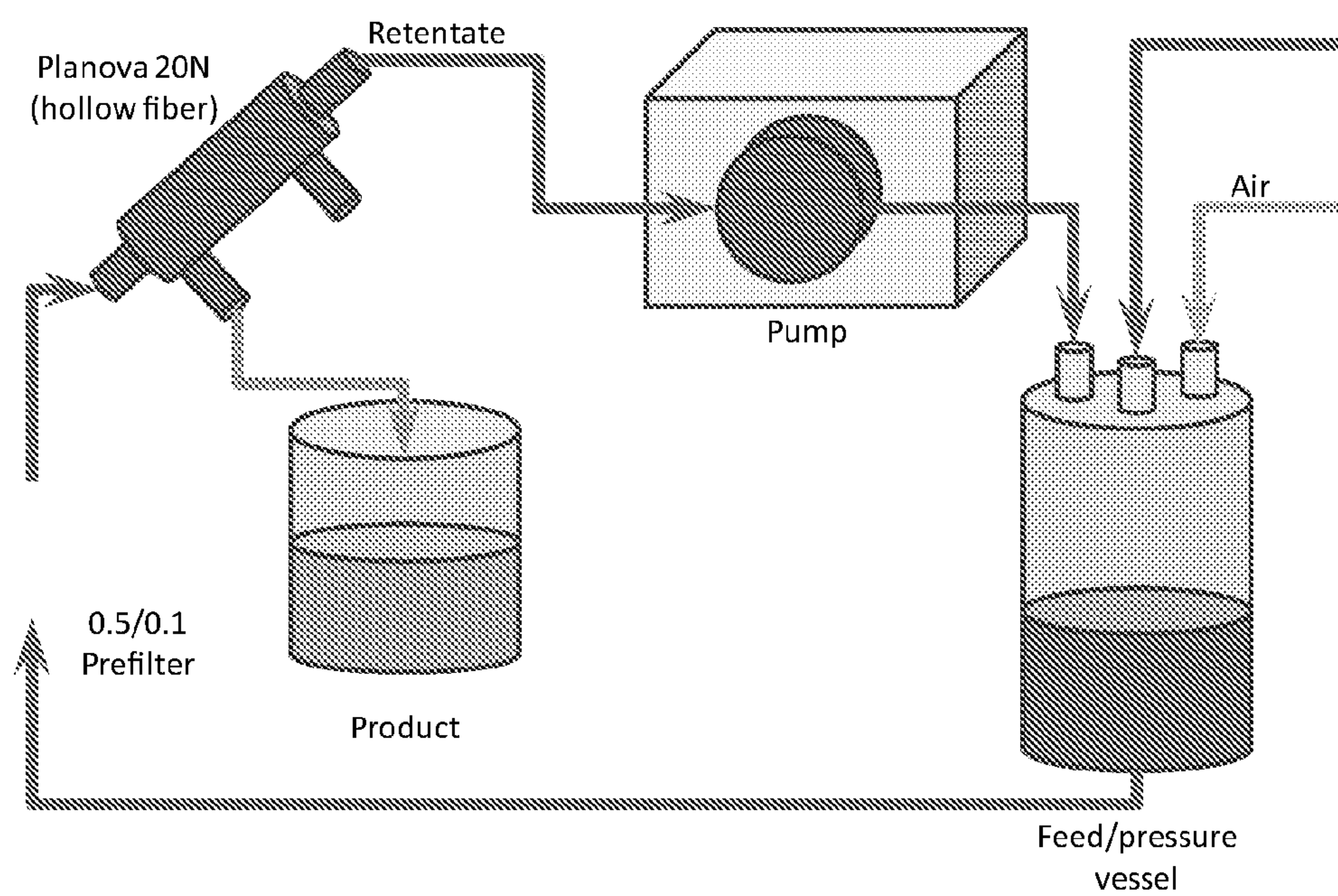


FIG. 11

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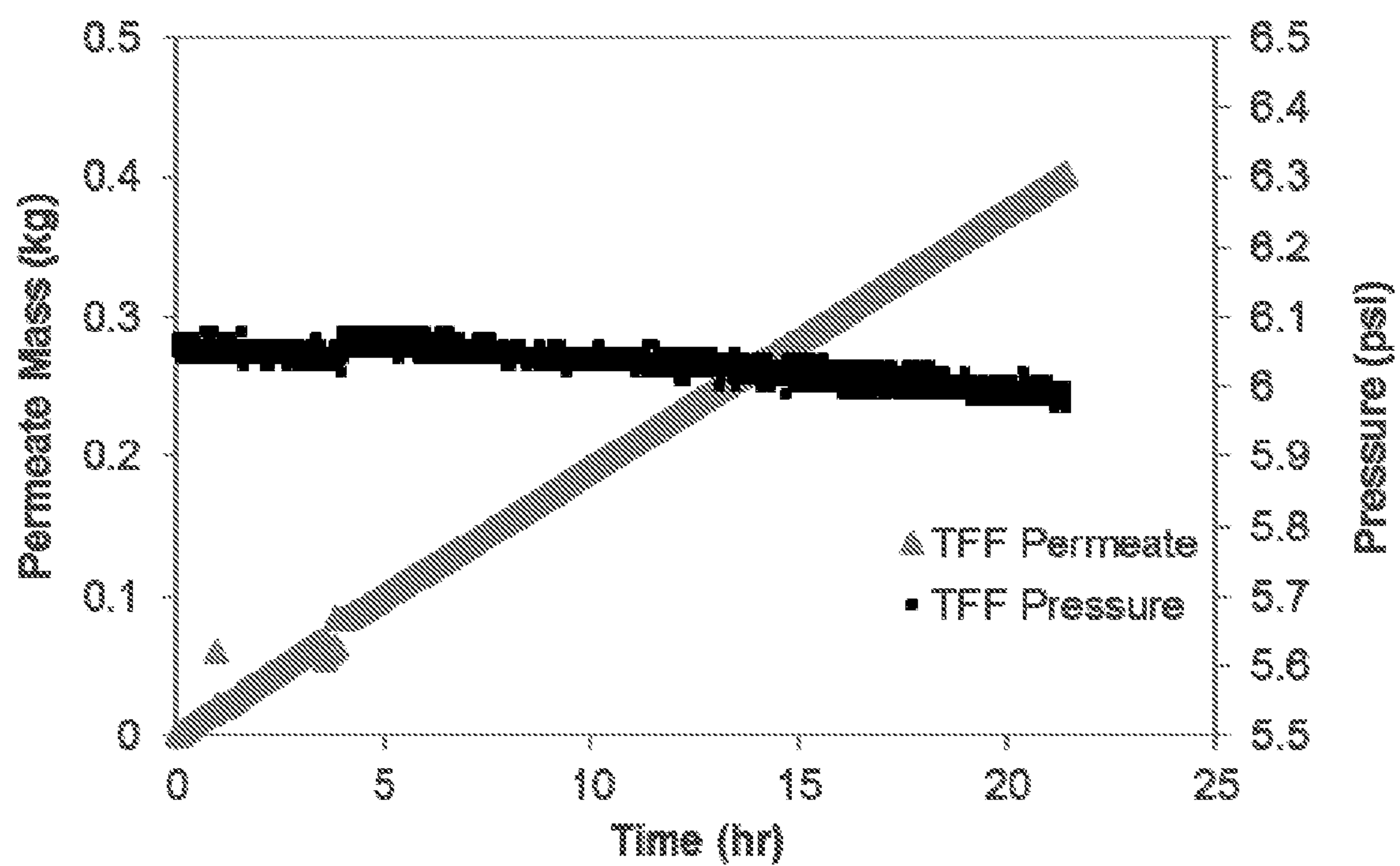


FIG. 12

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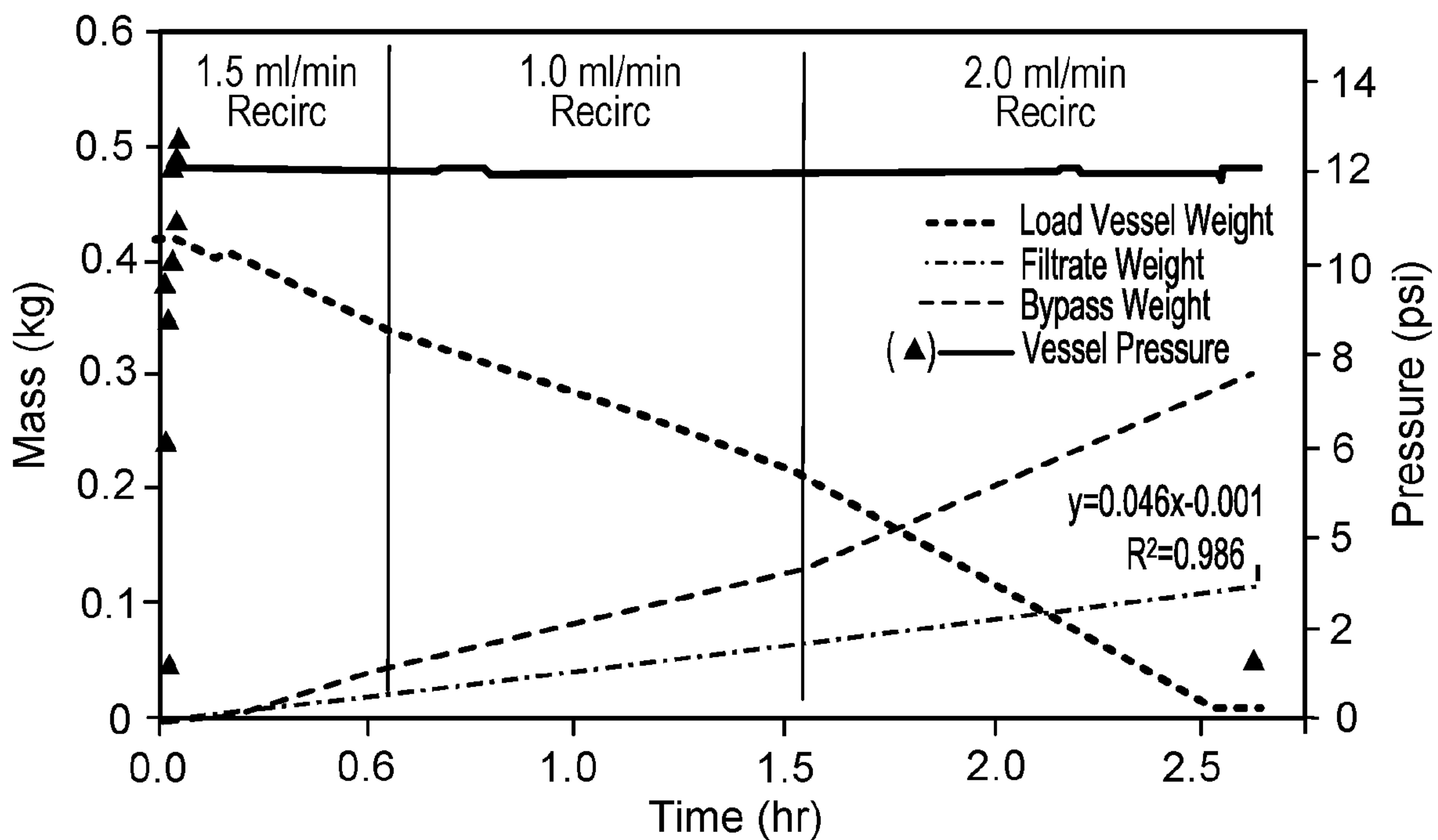


FIG. 13

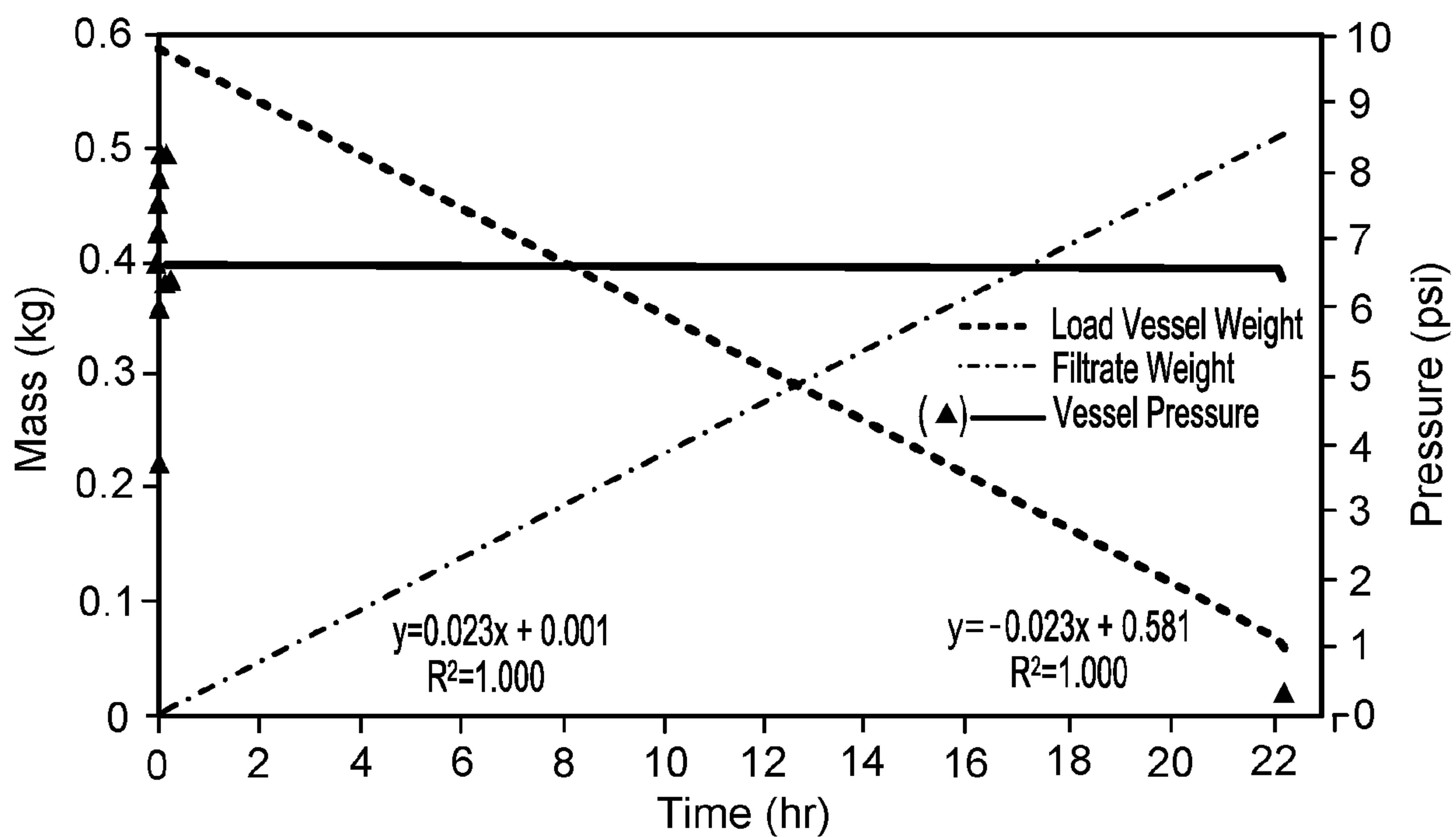
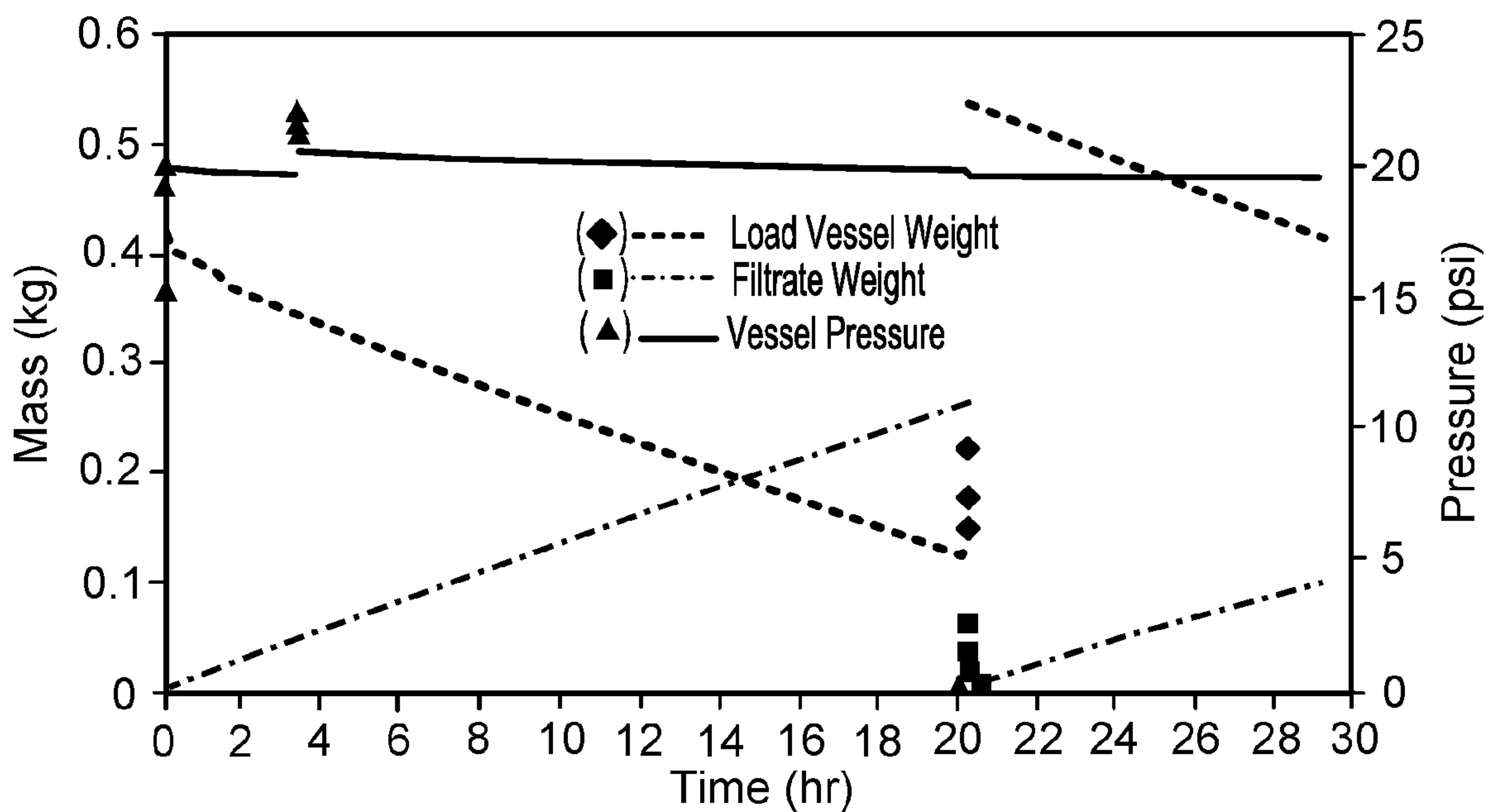
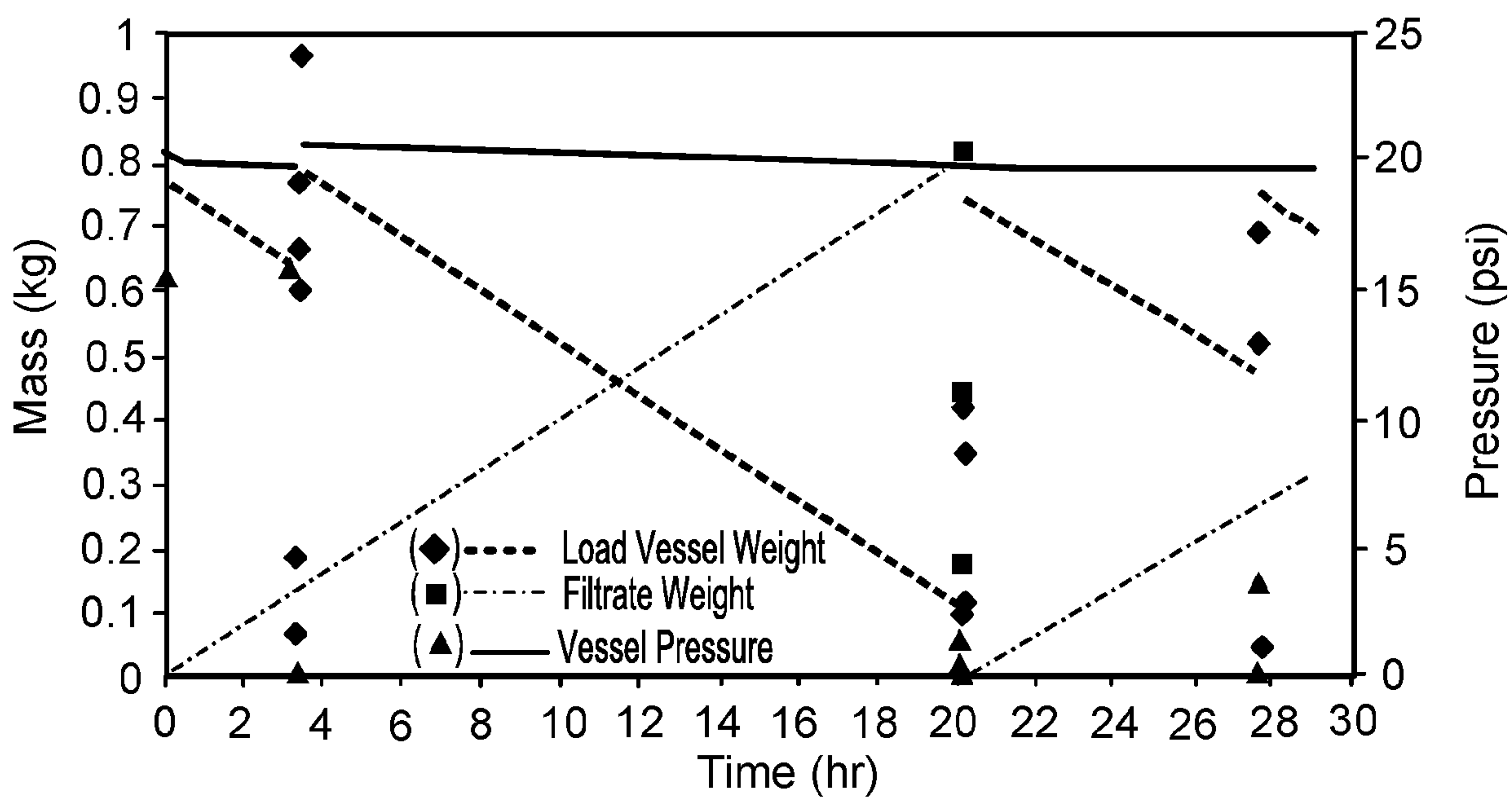


FIG. 14

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**FIG. 15****FIG. 16**

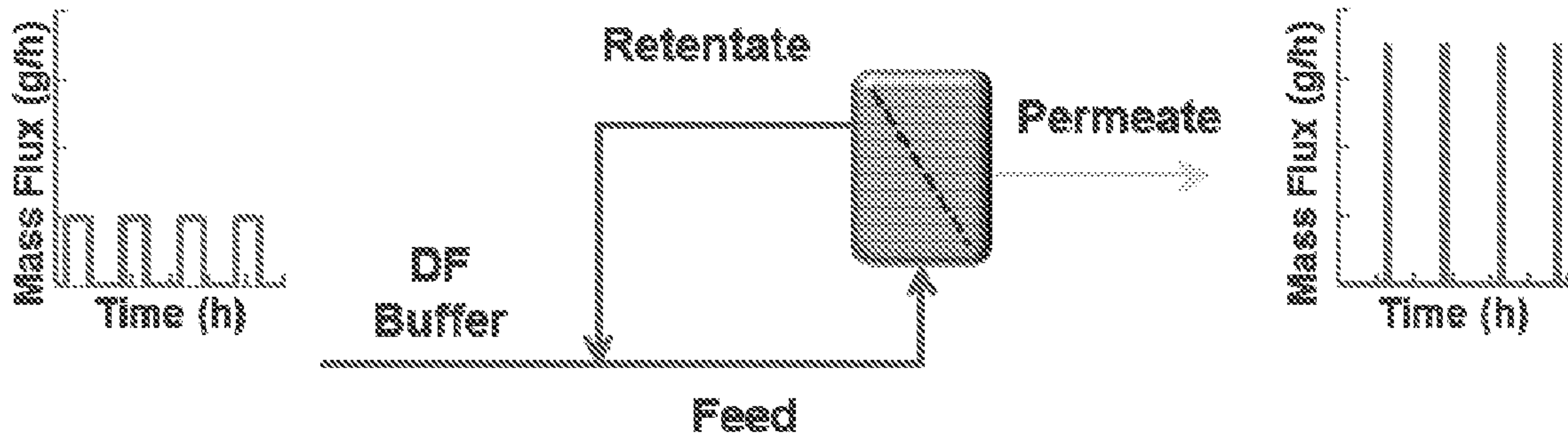


FIG. 6