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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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, 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.

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[Continued on next page]

(54) **Title**: METHODS OF CULTURING A MAMMALIAN CELL

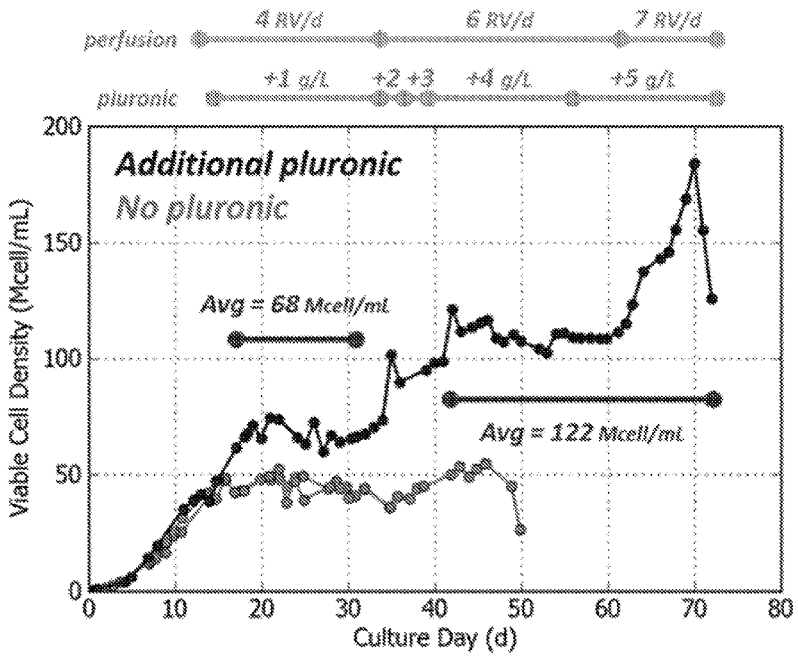

FIG. 1

[Continued on next page]

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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).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
3 November 2016

(57) Abstract: Provided herein are methods of culturing a mammalian cell in a liquid medium including poloxamer-188 at a concentration of 1.8 g/L or at a greater concentration than 1.8 g/L more or a liquid medium that includes a poloxamer-188 concentration that is selected based on one or more factors selected from the group of: pore size, pore type, gas flow rate, viable cell density in the medium, and markers related to cell stress.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/067040

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

8-11(completely); 1-7, 12-18, 32-52, 81-90(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/067040

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Karin Tördahl ET AL: "Study of a perfusion process of Chinese Hamster Ovary cells by ATF filtration in bioreactor Ovary cells by ATF filtration in bioreactor", , 11 September 2009 (2009-09-11), XP055274145, Retrieved from the Internet: URL:http://www.refinetech.com/media/downloads/kth-thesis-presentation-atf-perfusion-in-cho.pdf [retrieved on 2016-05-20] the whole document ----- -/--	1-4, 8-14,18, 32-36, 40-42, 46-52, 81-90

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 May 2016	05/09/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Manu, Dominique

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/067040

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	MANOHAR KATAKAM ET AL: "Effect of Surfactants on the Physical Stability of Recombinant Human Growth Hormone", JOURNAL OF PHARMACEUTICAL SCIENCES, AMERICAN PHARMACEUTICAL ASSOCIATION, WASHINGTON, US, vol. 84, no. 6, 1 June 1995 (1995-06-01), pages 713-716, XP002114576, ISSN: 0022-3549 the whole document -----	
X,P	WO 2015/188009 A1 (AMGEN INC [US]) 10 December 2015 (2015-12-10) the whole document -----	1-4, 12-14, 18, 32-36, 40-42, 46-52

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/067040

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015188009 A1	10-12-2015	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 8-11(completely); 1-7, 12-18, 32-52, 81-90(partially)

   method of perfusion culturing a mammalian cell expressing a recombinant protein in a medium comprising at least 1.8 g/L of poloxamer-188 and method claims relating to the ratio of antifoam to poloxamer-188

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2. claims: 19-31(completely); 1-7, 12-18, 32-54, 81-90(partially)

   method of perfusion culturing a mammalian cell expressing a recombinant protein in comprising adjusting the concentration of poloxamer-188 to the viable cell density

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3. claims: 55-75(completely); 53, 54, 76-90(partially)

   method of perfusion culturing a mammalian cell expressing a recombinant protein in a vessel comprising a porous sparger and in a medium comprising poloxamer-188 wherein the concentration of poloxamer-188 is selected based on pore size or pore types

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4. claims: 53, 54, 76-80(all partially)

   method of perfusion culturing a mammalian cell expressing a recombinant protein in a vessel comprising a porous sparger and in a medium comprising poloxamer-188 wherein the concentration of poloxamer-188 is selected based on gas flow rate

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5. claims: 53, 54, 76-90(all partially)

   method of perfusion culturing a mammalian cell expressing a recombinant protein in a vessel comprising a porous sparger and in a medium comprising poloxamer-188 wherein the concentration of poloxamer-188 is selected based on markers related to cell stress

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