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(54) Titre : COMPOSITION PHARMACEUTIQUE CONTENANT UN ANTICORPS ANTI-IL-5 ET SON UTILISATION
(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING ANTIBODY AGAINST IL-5 AND USE THEREOF

(57) Abrégé/Abstract:

Disclosed in the present application are a pharmaceutical composition containing an antibody against IL-5 and use thereof. In particular, disclosed in the present application is a pharmaceutical composition, which contains an IL-5 antibody or an antigen binding fragment thereof in a buffer solution. In addition, the pharmaceutical composition further contains a saccharide and a nonionic surfactant.



Abstract

Disclosed in the present application are a pharmaceutical composition containing an antibody against IL-5 and use thereof. In particular, disclosed in the present application is a
5 pharmaceutical composition, which contains an IL-5 antibody or an antigen binding fragment thereof in a buffer solution. In addition, the pharmaceutical composition further contains a saccharide and a nonionic surfactant.

PHARMACEUTICAL COMPOSITION CONTAINING ANTIBODY AGAINST IL-5 AND USE THEREOF

FIELD OF THE INVENTION

5 The present disclosure belongs to the field of pharmaceutical formulation, and in particular relates to a pharmaceutical composition comprising an anti-IL-5 antibody or an antigen-binding fragment thereof, and the use of the same as a diagnostic and therapeutic agent for IL-5 related disease(s).

BACKGROUND OF THE INVENTION

10 The statements herein only provide background information related to the present disclosure, and do not necessarily constitute the prior art.

Interleukin-5 (IL-5) is one of the important members of interleukin family, also known as T cell replacing factor (TRF), B cell growth factor-II (BCGF-II), IgA-enhancing factor (IgA-EF), or eosinophil differentiation factor (EDF) which is a homo-dimeric glycoprotein secreted mainly by helper T cells 2 (Th2). Human IL-5 is composed of 134 amino acid residues, including a signal peptide composed of 22 amino acids and two glycosylation sites. The active IL-5 is in a form of oligo-dimer, wherein two peptide chains in antiparallel configuration are linked by disulfide
15 bond(s); whereas the IL-5 monomer does not have biological activity (Adv Immunol. 1994; 57: 145-90).

Eosinophil (EOS) is related to a variety of inflammatory diseases of the lungs, including allergic diseases related to allergic reactions. Asthma is a chronic respiratory inflammatory disease.
25 There are about 300 million patients worldwide with an incidence rate of 10%. The pathogenesis of asthma is related to a variety of cytokines. IL-5 and the receptor IL-5R play an important role in the pathogenesis of asthma. At present, the most effective way to treat asthma is to administer

sterols by nasal or oral route, so as to inhibit the expression of several key mediators (including IL-5) involving in asthma and thereby reduce lung inflammation. However, long-term application of steroid agents has many side effects. Therefore, it is necessary to find novel pharmaceutical target for the treatment of asthma. Studies have shown that the administration of anti-IL-5 antibody inhibits the binding of IL-5 to its receptor; significantly reduces the accumulation of eosinophils in lung, and reduces the level of eosinophils in blood, tissues and sputum; reduces the inflammatory response mediated by eosinophils; improves lung function; and have a favorable effect on severe eosinophilic asthma and recurrent asthma (Drugs. 2017 May; 77(7):777-784).

Antibody agents have large molecular weight and complex structures. In the process of production, transportation and storage, antibodies often encounter problems due to denaturation, aggregation, contamination and formation of particles. In order to keep the antibody effective, the biological activity of antibody must be maintained during production, purification, transportation and storage. At present, new technologies for production and purification have been developed to produce large number of highly purified monoclonal antibodies. However, how to stabilize these antibodies during transportation and storage and to provide antibodies in dosage forms suitable for administration has been always a challenge.

For anti-IL-5 antibodies, only Mepolizumab from GSK and Reslizumab from Teva Pharma are currently approved for marketing. Related patents involve WO2018119016, WO2017033121, WO2014141149, WO2016040007, WO2015095539, WO2012138958 and WO9535375 etc.

SUMMARY OF THE INVENTION

The present disclosure provides a pharmaceutical composition which comprises an IL-5 antibody or an antigen-binding fragment thereof, a buffer and a surfactant, wherein the buffer is any one selected from the group consisting of acetic acid-sodium acetate, succinic acid-sodium succinate,

histidine-hydrochloride and citric acid-sodium citrate buffer, preferably acetic acid-sodium acetate or succinic acid-sodium succinate buffer; wherein, the anti-IL-5 antibody or the antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein:

- 5 (i) the heavy chain variable region comprises HCDR1, HCDR2 and HCDR3 as shown in amino acid sequence SEQ ID NOs: 16, 17 and 18, respectively, and
the light chain variable region comprises LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOs: 19, 20 and 21, respectively;
- (ii) the heavy chain variable region comprises HCDR1, HCDR2 and HCDR3 as shown in amino
10 acid sequence SEQ ID NOs: 22, 23 and 24, respectively, and
the light chain variable region comprises LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOs: 25, 26 and 27, respectively;
- (iii) the heavy chain variable region comprises HCDR1, HCDR2 and HCDR3 as shown in amino
acid sequence SEQ ID NOs: 28, 29 and 30, respectively, and
15 the light chain variable region comprises LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOs: 31, 32 and 33, respectively;
- (iv) the heavy chain variable region comprises HCDR1, HCDR2 and HCDR3 as shown in amino
acid sequence SEQ ID NOs: 34, 35 and 36, respectively, and
the light chain variable region comprises LCDR1, LCDR2 and LCDR3 as shown in amino acid
20 sequence SEQ ID NOs: 37, 38 and 39, respectively;
- (v) the heavy chain variable region comprises HCDR1, HCDR2 and HCDR3 as shown in amino
acid sequence SEQ ID NOs: 40, 41 and 42, respectively, and
the light chain variable region comprises LCDR1, LCDR2 and LCDR3 as shown in amino acid
sequence SEQ ID NOs: 43, 44 and 45, respectively; or
- 25 (vi) the heavy chain variable region comprises HCDR1, HCDR2 and HCDR3 as shown in amino
acid sequence SEQ ID NOs: 34, 82 and 36, respectively, and
the light chain variable region comprises LCDR1, LCDR2 and LCDR3 as shown in amino acid

sequence SEQ ID NOs: 37, 38 and 39, respectively.

In alternative embodiments, the pH of the buffer in the pharmaceutical composition is about 5.0 to about 6.5, preferably about 5.5 to about 6.5, preferably about 6.0 to about 6.5, preferably about 5.0 to about 6.0, preferably about 5.5 to about 6.0, preferably about 5.0 to about 5.5, preferably about 5.0 to about 5.8, preferably about 5.2 to about 5.8; non-limiting examples of the pH of the buffer comprise about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.5, and most preferably about 5.5.

10 In alternative embodiments, the concentration of the buffer in the pharmaceutical composition is about 10mM to about 40mM, about 10mM to about 30mM, preferably about 15mM to about 30mM, preferably about 20mM to about 30mM, preferably about 25mM to about 30mM, preferably about 10mM to about 25mM, preferably about 15mM to about 25mM, preferably about 20mM to about 25mM, preferably about 10mM to about 15mM; non-limiting examples of
15 the concentration of the buffer involve about 10mM, about 12mM, about 14mM, about 16mM, about 18mM, about 20mM, about 22mM, about 24mM, about 26mM, about 28mM, about 30mM, about 32mM, about 34mM, and most preferably about 30mM.

In alternative embodiments, the concentration of the anti-IL-5 antibody or the antigen-binding
20 fragment thereof in the pharmaceutical composition is about 1 mg/ml to about 120 mg/ml, preferably about 1 mg/ml to about 100 mg/ml, preferably about 10 mg/ml to about 120 mg/ml, preferably about 20 mg/ml to about 120 mg/ml, preferably about 30 mg/ml to about 120 mg/ml, preferably about 40 mg/ml to about 120 mg/ml, preferably about 50 mg/ml to about 120 mg/ml, preferably about 60 mg/ml to about 120 mg/ml, preferably about 70 mg/ml to about 120 mg/ml,
25 preferably about 80 mg/ml to about 120 mg/ml, preferably about 90 mg/ml to about 120 mg/ml, preferably about 100 mg/ml to about 120 mg/ml, preferably about 110 mg/ml to about 120 mg/ml, preferably about 20 mg/ml to about 100 mg/ml, preferably about 30 mg/ml to about 100

mg/ml, preferably about 40 mg/ml to about 100 mg/ml, preferably about 50 mg/ml to about 100 mg/ml, preferably about 60 mg/ml to about 100 mg/ml, preferably about 70 mg/ml to about 100 mg/ml, preferably about 80 mg/ml to about 100 mg/ml, preferably about 90 mg/ml to about 100 mg/ml; as non-limiting examples, the concentration of the anti-IL-5 antibody or the antigen-binding fragment thereof is about 80 mg/ml, about 85 mg/ml, about 90 mg/ml, about 91 mg/ml, about 92 mg/ml, about 93 mg/ml, about 94 mg/ml, about 95 mg/ml, about 96 mg/ml, about 97 mg/ml, about 98 mg/ml, about 99 mg/ml, about 100 mg/ml, about 101 mg/ml, about 102 mg/ml, about 103 mg/ml, about 104 mg/ml, about 105 mg/ml, about 106 mg/ml, about 107 mg/ml, about 108 mg/ml, about 109 mg/ml, about 110 mg/ml, about 115 mg/ml, about 120 mg/ml, and most preferably about 100 mg/ml.

In alternative embodiments, the surfactant comprised in the pharmaceutical composition can be selected from the group consisting of polysorbate 20, polysorbate 80, polyhydroxyalkylene, Triton, sodium dodecyl sulfonate, sodium lauryl sulfonate, sodium octyl glycoside, lauryl-sulphobetaine, myristyl-sulphobetaine, linoleyl-sulphobetaine, stearyl-sulphobetaine, lauryl-sarcosine, myristyl-sarcosine, linoleyl-sarcosine, stearyl-sarcosine, linoleyl-betaine, myristyl-betaine, cetyl-betaine, lauramidopropyl-betaine, cocamidopropyl-betaine, linoleamidopropyl-betaine, myristamidopropyl-betaine, palmitamidopropyl-betaine, isosteamidopropyl-betaine, myristamidopropyl-dimethylamine, palmamidopropyl-dimethylamine, isostearamidopropyl-dimethylamine, sodium methylcocoyl, sodium methyl-oleoyl taurate, polyethylene glycol, polypropylene glycol, copolymers of ethylene and propylene glycol, etc. The preferred surfactant is polysorbate 80 or polysorbate 20, and more preferably polysorbate 80.

In alternative embodiments, the concentration of polysorbate 80 in the pharmaceutical composition is about 0.05 mg/ml to about 0.6 mg/ml, preferably about 0.1 mg/ml to about 0.6 mg/ml, preferably about 0.2 mg/ml to about 0.6 mg/ml, preferably about 0.3 mg/ml to about 0.6

mg/ml, preferably about 0.4 mg/ml to about 0.6 mg/ml, preferably about 0.5 mg/ml to about 0.6 mg/ml, preferably about 0.2 mg/ml to about 0.5 mg/ml, preferably about 0.3 mg/ml to about 0.5 mg/ml, preferably about 0.4 mg/ml to about 0.5 mg/ml, preferably about 0.3 mg/ml to about 0.4 mg/ml, as non-limiting examples, the concentration of the surfactant in the pharmaceutical composition is about 0.2 mg/ml, about 0.3 mg/ml, about 0.4 mg/ml, about 0.45 mg/ml, about 0.5 mg/ml, about 0.55 mg/ml, about 0.6 mg/ml, and most preferably about 0.4 mg/ml.

Further, in alternative embodiments, the pharmaceutical composition further comprises excipient(s), wherein the excipient is selected from stabilizers.

In alternative embodiments, wherein the stabilizer is selected from saccharide or amino acid; wherein the saccharide can be selected from the group consisting of sucrose, trehalose, mannitol and sorbitol, preferably sucrose. The amino acid is selected from the group consisting of glycine, methionine and proline.

In alternative embodiments, the concentration of the saccharide is about 50 mg/ml to about 80 mg/ml, preferably about 60 mg/ml to about 80 mg/ml, preferably about 70 mg/ml to about 80 mg/ml, preferably about 75 mg/ml to about 80 mg/ml, preferably about 70 mg/ml to about 75 mg/ml; as non-limiting examples, the concentration of the stabilizer in the pharmaceutical composition involves about 70 mg/ml, about 71 mg/ml, about 72 mg/ml, about 73 mg/ml, about 74 mg/ml, about 75 mg/ml, about 76 mg/ml, about 77 mg/ml, about 78 mg/ml, about 79 mg/ml, about 80 mg/ml, and more preferably about 72 mg/ml.

In alternative embodiments, the concentration of the amino acid is about 8 mg/ml.

In alternative embodiments, the pharmaceutical composition comprises:

(a) about 1 mg/ml to about 120 mg/ml the anti-IL-5 antibody or the antigen-binding fragment

thereof; (b) about 10mM to about 30mM acetic acid-sodium acetate buffer, pH is about 5.0 to 6.5; and (c) about 0.1 mg/ml to about 0.6 mg/ml polysorbate 80.

In alternative embodiments, the pharmaceutical composition comprises:

- 5 (a) about 80 mg/ml to about 100 mg/ml the anti-IL-5 antibody or the antigen-binding fragment thereof; (b) about 10mM to about 30mM acetic acid-sodium acetate buffer, pH is about 5.0 to about 6.0; and (c) about 0.1 mg/ml to about 0.4 mg/ml polysorbate 80.

In alternative embodiments, the pharmaceutical composition comprises:

- 10 (d) about 80 mg/ml to about 120 mg/ml the IL-5 antibody or the antigen-binding fragment thereof; (e) about 10mM to about 30mM acetic acid-sodium acetate buffer, pH is about 5.0 to about 5.8; (f) about 0.2 mg/ml to about 0.6 mg/ml polysorbate 80; and (g) about 70 mg/ml to about 75 mg/ml sucrose; preferably, the pharmaceutical composition preferably comprises:
(h) about 100 mg/ml the IL-5 antibody or the antigen-binding fragment thereof, (i) about 30mM
15 acetic acid-sodium acetate buffer, pH is about 5.5, (j) about 0.4 mg/ml polysorbate 80 and (k) about 72 mg/ml of sucrose.

- In some preferred embodiments, the anti-IL-5 antibody or the antigen-binding fragment thereof in the pharmaceutical composition of the present disclosure is a murine antibody, a chimeric
20 antibody or a humanized antibody.

- In alternative embodiments, the humanized anti-IL-5 antibody in the pharmaceutical composition comprises a heavy chain variable region as shown in SEQ ID NO: 49, 57, 63, 69 or 75 or variant thereof; the variant comprises 1 to 10 amino acid back-mutations in the heavy chain variable
25 region sequence as shown in SEQ ID NO: 49, 57, 63, 69 or 75, respectively.

In alternative embodiments, the variant is a variant as shown in any one selected from the group consisting of:

- (i) a variant, comprising one or more amino acid back-mutations selected from the group consisting of S49T, V93T and K98S in the heavy chain variable region as shown in SEQ ID NO: 49;
- (ii) a variant, comprising one or more amino acid back-mutations selected from the group consisting of S49T, V93T and K98T in the heavy chain variable region as shown in SEQ ID NO: 57;
- (iii) a variant, comprising one or more amino acid back-mutations selected from the group consisting of R38K, M48I, R67K, V68A, M70L, R72V, T74K and L83F in the heavy chain variable region as shown in SEQ ID NO: 63;
- (iv) a variant, comprising one or more amino acid back-mutations selected from the group consisting of F29I, R38K, V48I, R72A, and T97F in the heavy chain variable region as shown in SEQ ID NO: 69, and/or N55V mutation in CDR; or
- (v) a variant, comprising one or more amino acid back-mutations selected from the group consisting of R38K, M48I, R67K, V68A, R72A, T74K, M81L, L83F and D89E in the heavy chain variable region as shown in SEQ ID NO: 75.

In alternative embodiments, the humanized anti-IL-5 antibody in the pharmaceutical composition comprises:

- a heavy chain variable region as shown in SEQ ID NO: 50 or 51; or
- a heavy chain variable region as shown in SEQ ID NO: 58 or 59; or
- a heavy chain variable region as shown in any one selected from the group consisting of: SEQ ID NO: 64, 65 and 66; or
- a heavy chain variable region as shown in SEQ ID NO: 70 or 71; or
- a heavy chain variable region as shown in any one selected from the group consisting of: SEQ ID NO: 76, 77, 78 and 79.

In alternative embodiments, the humanized anti-IL-5 antibody in the pharmaceutical composition comprises a light chain variable region as shown in SEQ ID NO: 46, 54, 60, 67 or 72, or variants thereof; the variant comprises 1 to 10 amino acid back-mutations in the light chain variable
 5 region as shown in SEQ ID NO: 46, 54, 60, 67 or 72.

In alternative embodiments, wherein the variant is a variant as shown in any one selected from the group consisting of:

- (i) a variant, comprising one or more amino acid back-mutations selected from the group
 10 consisting of A43S, L47V, G66R, T69S, F71Y and Y87F in the light chain variable region as shown in SEQ ID NO: 46;
- (ii) a variant, comprising one or more amino acid back-mutations selected from the group consisting of A43S, L47M, F71Y and Y87F in the light chain variable region as shown in SEQ ID NO: 54;
- 15 (iii) a variant, comprising amino acid back-mutation(s) selected from the group consisting of E1D, I2T, I57V, V84T and Y86F in the light chain variable region as shown in SEQ ID NO: 60, or the combination thereof;
- (iv) a variant, comprising one or more amino acid back-mutations selected from the group consisting of: M4L, A42S, L45P and L46W in the light chain variable region as shown in SEQ
 20 ID NO: 67; and
- (v) a variant, comprising one or more amino acid back-mutations selected from the group consisting of A43S, I48V and F71Y in the light chain variable region as shown in SEQ ID NO: 72.

25 In alternative embodiments, the humanized anti-IL-5 antibody in the pharmaceutical composition comprises:
 a light chain variable region as shown in SEQ ID NO: 47 or 48; or

a light chain variable region as shown in SEQ ID NO: 55 or 56; or
 a light chain variable region as shown in SEQ ID NO: 61 or 62; or
 a light chain variable region as shown in SEQ ID NO: 68; or
 a light chain variable region as shown in SEQ ID NO: 73 or 74.

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In alternative embodiments, the humanized anti-IL-5 antibody in the pharmaceutical composition comprises:

- (i) a heavy chain variable region as shown in any one of SEQ ID NO: 49, 50 and 51 or having 95% sequence identity with any one of SEQ ID NO: 49, 50 and 51; and
- 10 a light chain variable region as shown in any one of SEQ ID NO: 46, 47 and 48 or having 95% sequence identity with any one of SEQ ID NO: 46, 47 and 48;
- (ii) a heavy chain variable region as shown in any one of SEQ ID NO: 57, 58 and 59 or having 95% sequence identity with any one of SEQ ID NO: 57, 58 and 59; and
- a light chain variable region as shown in any one of SEQ ID NO: 54, 55 and 56 or having 95% sequence identity with any one of SEQ ID NO: 54, 55 and 56;
- 15 (iii) a heavy chain variable region as shown in any one of SEQ ID NO: 63, 64, 65 and 66 or having 95% sequence identity with any one of SEQ ID NO: 63, 64, 65 and 66; and
- a light chain variable region as shown in any one of SEQ ID NO: 60, 61 and 62 or having 95% sequence identity with any one of SEQ ID NO: 60, 61 and 62;
- 20 (iv) a heavy chain variable region as shown in any one of SEQ ID NO: 69, 70 and 71 or having 95% sequence identity with any one of SEQ ID NO: 69, 70 and 71; and
- a light chain variable region as shown in any one of SEQ ID NO: 67 and 68 or having 95% sequence identity with any one of SEQ ID NO: 67 and 68; or
- (v) a heavy chain variable region as shown in any one of SEQ ID NO: 75, 76, 77, 78 and 79 or
- 25 having 95% sequence identity with any one of SEQ ID NO: 75, 76, 77, 78 and 79; and
- a light chain variable region as shown in any one of SEQ ID NO: 72, 73 and 74 or having 95% sequence identity with any one of SEQ ID NO: 72, 73 and 74; preferably, the humanized

anti-IL-5 antibody comprises:

- (a) the heavy chain variable region as shown in SEQ ID NO: 51 and the light chain variable region as shown in SEQ ID NO: 47;
- (b) the heavy chain variable region as shown in SEQ ID NO: 65 and the light chain variable region as shown in SEQ ID NO: 62;
- (c) the heavy chain variable region as shown in SEQ ID NO: 58 and the light chain variable region as shown in SEQ ID NO: 56;
- (d) the heavy chain variable region as shown in SEQ ID NO: 71 and the light chain variable region as shown in SEQ ID NO: 68; or
- (e) the heavy chain variable region as shown in SEQ ID NO: 79 and the light chain variable region as shown in SEQ ID NO: 73.

The amino acid sequence having at least 95% sequence identity as described above, preferably has at least 95%, 96%, 97%, 98% or 99% sequence identity, and more preferably has 97%, 98% or 99% or above, and most preferably has at least 99% sequence identity or above, the amino acid sequence having at least 95% sequence identity as described above comprises one or more amino acid deletions, insertions or substitutions obtained by mutation.

In alternative embodiments, the anti-IL-5 antibody in the pharmaceutical composition comprises a human antibody constant region, preferably a human antibody heavy chain constant region as shown in SEQ ID NO: 52 and a human antibody light chain constant region as shown in SEQ ID NO: 53.

In alternative embodiments, the anti-IL-5 antibody in the pharmaceutical composition comprises:

- (i) a heavy chain as shown in SEQ ID NO: 83 and a light chain as shown in SEQ ID NO: 84;
- (ii) a heavy chain as shown in SEQ ID NO: 85 and a light chain as shown in SEQ ID NO: 86;
- (iii) a heavy chain as shown in SEQ ID NO: 87 and a light chain as shown in SEQ ID NO: 88;

- (iv) a heavy chain as shown in SEQ ID NO: 89 and a light chain as shown in SEQ ID NO: 90; or
- (v) a heavy chain as shown in SEQ ID NO: 91 and a light chain as shown in SEQ ID NO: 92.

In alternative embodiments, the anti-IL-5 antibody in the pharmaceutical composition is a
 5 monoclonal antibody or antigen-binding fragment thereof that competes for binding to IL-5 with
 the anti-IL-5 antibody or the antigen-binding fragment thereof as described above.

In a preferred embodiment, the antigen-binding fragment in the pharmaceutical composition of
 the present disclosure is selected from the group consisting of Fab, Fab', F(ab')₂, single-chain
 10 antibody (scFv), dimerized V region (diabody) and disulfide bond stabilized V region (dsFv).

The present disclosure further provides a method for preparing the pharmaceutical composition
 as described above, wherein it comprises a step of replacing a stock solution of the anti-IL-5
 antibody with a buffer. In alternative embodiments, preferably the buffer is acetic acid-sodium
 15 acetate buffer. The pH of the buffer is about 5.0 to about 6.5, preferably about 5.5 to about 6.5,
 preferably about 6.0 to about 6.5, preferably about 5.0 to about 6.0, preferably about 5.5 to about
 6.0, preferably about 5.0 to about 5.5, preferably about 5.2 to about 5.8, non-limiting examples
 of the buffer pH value involve about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5,
 about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.5, and most preferably about 5.5. In
 20 alternative embodiments, the concentration of the buffer in the pharmaceutical composition is
 about 10mM to about 30mM, preferably about 15mM to about 30mM, preferably about 20mM to
 about 30mM, preferably about 25mM to about 30mM, preferably about 5mM to about 25mM,
 preferably about 10mM to about 25mM, preferably about 15mM to about 25mM, preferably
 about 20mM to about 25mM, preferably about 5mM to about 20mM, preferably about 10mM to
 25 about 15mM; non-limiting examples of the concentration of the buffer involve about 10mM,
 about 12mM, about 14mM, about 16mM, about 18mM, about 20mM, about 22mM, about 24mM,
 about 26mM, about 28mM, about 30mM, and most preferably about 30mM.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate, pH 5.5 and 0.2 mg/mL polysorbate 80.

- 5 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate, pH 5.5 and 0.05 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 5.0 and 0.2 mg/mL polysorbate 80.

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In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 5.5 and 0.2 mg/mL polysorbate 80.

- 15 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 6.0 and 0.2 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 5.0 and 0.05 mg/mL polysorbate 80.

- 20 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 5.5 and 0.05 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 6.0 and 0.05 mg/mL polysorbate 80.

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In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM citric acid-sodium citrate, pH 6.5 and 0.2 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM citric acid-sodium citrate, pH 5.5 and 0.2 mg/mL polysorbate 80.

- 5 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM citric acid-sodium citrate, pH 6.0 and 0.2 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM citric acid-sodium citrate, pH 6.5 and 0.05 mg/mL polysorbate 80.

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- 20 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM citric acid-sodium citrate, pH 5.5 and 0.2 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM histidine-hydrochloric acid, pH 6.0 and 0.2 mg/mL polysorbate 80.

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In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM histidine-hydrochloric acid, pH 6.5 and 0.05 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM histidine-hydrochloric acid, pH 5.5 and 0.05 mg/mL polysorbate 80.

- 5 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM histidine-hydrochloric acid, pH 6.0 and 0.05 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 5.0 and 0.1 mg/mL polysorbate 20.

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In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate, pH 5.0 and 0.1 mg/mL polysorbate 80.

- 15 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate pH 5.0, 50 mg/mL sucrose and 0.1 mg/mL polysorbate 80.

- 20 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate pH 5.0, 50 mg/mL trehalose and 0.1 mg/mL polysorbate 80.

- In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate pH 5.0, 50 mg/mL mannitol and 0.1 mg/mL polysorbate 80.

25

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate pH 5.0, 50 mg/mL sorbitol and 0.1 mg/mL

polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate pH 5.0, 8 mg/mL glycine and 0.1 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10 mM succinic acid-sodium succinate pH 5.0, 8 mg/mL methionine and 0.1 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10 mM succinic acid-sodium succinate pH 5.0, 8 mg/mL proline and 0.1 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM succinic acid-sodium succinate pH 5.5, 70 mg/mL sucrose and 0.4 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 80 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.8 and 0.2 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.4 and 0.6 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 80 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.4 and 0.4 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 80 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.0 and 0.2 mg/mL polysorbate 80.

5 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.4 and 0.2 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 80 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH5.8 and 0.6 mg/mL polysorbate 80.

10 In some embodiments, the pharmaceutical composition comprises: 120 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.0 and 0.2 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 80 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.0 and 0.6 mg/mL polysorbate 80.

15

In some embodiments, the pharmaceutical composition comprises: 120 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.8 and 0.6 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 120 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.4 and 0.4 mg/mL polysorbate 80.

20

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.4 and 0.4 mg/mL polysorbate 80.

25 In some embodiments, the pharmaceutical composition comprises: 120 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.0 and 0.6 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 120 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.8 and 0.2 mg/mL polysorbate 80.

5 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.0 and 0.4 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.8 and 0.4 mg/mL polysorbate 80.

10 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 10mM acetic acid-sodium acetate pH 5.5, 70 mg/ml sucrose and 0.4 mg/mL polysorbate 80.

15 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 20mM acetic acid-sodium acetate pH 5.5, 70 mg/ml sucrose and 0.4 mg/mL polysorbate 80.

20 In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 30mM acetic acid-sodium acetate pH 5.5, 70 mg/ml sucrose and 0.4 mg/mL polysorbate 80.

In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 30mM acetic acid-sodium acetate pH 5.5, 73 mg/ml sucrose and 0.4 mg/mL polysorbate 80.

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In some embodiments, the pharmaceutical composition comprises: 100 mg/ml anti-IL-5 antibody h1705-008, 30mM acetic acid-sodium acetate pH 5.5, 75 mg/ml sucrose and 0.4 mg/mL

polysorbate 80.

In some embodiments, the pharmaceutical composition of the present disclosure is stable at 2-8°C for at least 3 months, at least 6 months, at least 12 months, at least 18 months, or at least 24 months. The pharmaceutical composition can be stable at 25°C for at least 3 months, at least 6 months.

The present disclosure further provides a method for preparing a lyophilized formulation comprising an anti-IL-5 antibody, which comprises a step of lyophilizing the pharmaceutical composition as described above.

In an alternative embodiment, the lyophilization in the method for preparing the lyophilized formulation comprising an anti-IL-5 antibody comprises the steps of pre-freezing, primary drying and secondary drying, successively. The lyophilization is carried out by freezing the formulation and subsequently sublimating the water at a temperature suitable for primary drying. Under such condition, the temperature of the product is lower than the eutectic point or decomposition temperature of the formulation. Under a suitable pressure, typically in the range of about 50 to 250 mTorr, the storage temperature for primary drying is usually about -30 to 25°C (assuming that the product remains frozen during the primary drying). The formulation, the size and type of sample container (for example, glass vial) and the volume of liquid determine the time duration required for drying, and the time duration can range from several hours to several days (for example, 40 to 60 hours). The secondary drying can be carried out at about 0 to 40°C, which mainly depends on the type and size of the container and the type of protein used. The time duration for secondary drying is determined by the desired residual humidity level of the product, and usually requires at least about 5 hours. Generally, the water content in lyophilized formulation prepared under low-pressure is less than about 5%, preferably less than about 3%. The pressure can be the same as the pressure applied in the primary drying step;

preferably the pressure used in the secondary drying is lower than that used in the primary drying. The conditions for lyophilization can vary with the formulation and vial size.

5 The present disclosure further provides a lyophilized formulation comprising an IL-5 antibody prepared by the method for preparing a lyophilized formulation comprising an anti-IL-5 antibody as described above.

10 In some embodiments, the lyophilized formulation is stable at 2-8°C for at least 3 months, at least 6 months, at least 12 months, at least 18 months or at least 24 months. In some embodiments, the lyophilized formulation is stable at 40°C for at least 7 days, at least 14 days or at least 28 days.

15 The present disclosure further provides a method for preparing a reconstituted solution of the lyophilized formulation comprising an anti-IL-5 antibody, wherein it comprises a step of reconstituting the lyophilized formulation as described above, and the solution used for the reconstitution comprises, but not limited to, water for injection, normal saline or glucose solution.

20 The present disclosure further provides a reconstituted solution of the lyophilized formulation comprising an IL-5 antibody prepared by the method for preparing a reconstituted solution of the lyophilized formulation comprising an anti-IL-5 antibody as described above.

25 The present disclosure further provides an article or kit which comprises container(s) comprising any of the stable pharmaceutical compositions herein. In some embodiments, the container is an injection vial made of neutral borosilicate glass.

The present disclosure further provides an article, which comprises container(s) comprising the pharmaceutical composition or the lyophilized formulation or the reconstituted solution of lyophilized formulation as described above.

- 5 The present disclosure further provides a method for treating IL-5 mediated disease(s), comprising administering a therapeutically effective amount of the pharmaceutical composition or the lyophilized formulation or the reconstituted solution or the article of manufacture as described above, to a subject in need thereof; wherein the IL-5 mediated disease is preferably selected from the group consisting of asthma, chronic pneumonia, allergic rhinitis, allergic
10 bronchopulmonary aspergillosis disease, eosinophilia, Churg-Strauss syndrome, atopic dermatitis, onchocerciasis dermatitis, intermittent angioedema, eosinophilic myalgia syndrome, eosinophilic gastroenteritis, worm infection, Hodgkin's disease, nasal polyps, Loeffler's syndrome, urticaria, hypereosinophilic bronchitis, nodular arteritis, sinusitis, eosinophilic esophagitis, allergic eosinophilic esophagitis, allergic conjunctivitis, onchocerciasis dermatitis, endometriosis and
15 steroid-dependent eosinophilic bronchitis.

The present disclosure further provides the use of the pharmaceutical composition or the lyophilized formulation or the reconstituted solution of the lyophilized formulation or the article of manufacture as described above in the preparation of a medicament for treating IL-5 mediated
20 disease(s); wherein the IL-5 mediated disease is preferably selected from the group consisting of asthma, chronic pneumonia, allergic rhinitis, allergic bronchopulmonary aspergillosis disease, eosinophilia, Churg-Strauss syndrome, atopic dermatitis, onchocerciasis dermatitis, intermittent angioedema, eosinophilic myalgia syndrome, eosinophilic gastroenteritis, worm infection, Hodgkin's disease, nasal polyps, Loeffler's syndrome, urticaria, hypereosinophilic bronchitis,
25 nodular arteritis, sinusitis, eosinophilic esophagitis, allergic eosinophilic esophagitis, allergic conjunctivitis, onchocerciasis dermatitis, endometriosis and steroid-dependent eosinophilic bronchitis.

The present disclosure further provides the pharmaceutical composition or the lyophilized formulation or the reconstituted solution of the lyophilized formulation or the article of manufacture as described above, for use as a therapeutic medicament, wherein the medicament is for treating IL-5 mediated disease(s); wherein the IL-5 mediated disease is preferably selected from the group consisting of asthma, chronic pneumonia, allergic rhinitis, allergic bronchopulmonary aspergillosis disease, eosinophilia, Churg-Strauss syndrome, atopic dermatitis, onchocerciasis dermatitis, intermittent angioedema, eosinophilic myalgia syndrome, eosinophilic gastroenteritis, worm infection, Hodgkin's disease, nasal polyps, Loeffler's syndrome, urticaria, hypereosinophilic bronchitis, nodular arteritis, sinusitis, eosinophilic esophagitis, allergic eosinophilic esophagitis, allergic conjunctivitis, onchocerciasis dermatitis, endometriosis and steroid-dependent eosinophilic bronchitis.

As is well known to those skilled in the art, one, some or all of the features of each embodiment in the present disclosure can be further combined to form other embodiments of the present disclosure. The embodiments of the present disclosure as described above and additional embodiments obtained by combination are further illustrated by the detailed description below.

DESCRIPTION OF THE DRAWINGS

Figure 1 represents the results of FACS experiments in which anti-IL-5 antibodies block the binding of IL-5 to IL-5 receptor;

Figure 2 represents the detection result of binding specificity of the anti-IL-5 antibody for Th2 cytokine;

Figure 3 represents that the anti-IL-5 antibody enhances the level of intermittent respiratory (Penh). G1: normal control group (PBS); G2: model group (IgG); G3: h1705-008 antibody

10mpk group; G4: h1705-008 antibody 2mpk group; G5: h1706-009 antibody 10mpk group; G6: h1706-009 antibody 2mpk group; G7: Hu39D10 10mpk group; wherein, * $p < 0.05$, ** $p < 0.01$ (compared with G2 group, by ANOVA/Bonferroni);

5 Figure 4A represents the level of BALF eosinophils in lungs of asthmatic mice; Figure 4B represents the tracheal mucosal thickness score of asthmatic mice. G1: normal control group; G2: model group; G3: h1705-008 antibody 10mpk group; G4: h1705-008 antibody 2mpk group; G5: h1706-009 antibody 10mpk group; G6: h1706-009 antibody 2mpk group; G7: Hu39D10 10mpk group; Figure 4C represents the percentage of BALF eosinophils in lungs of asthmatic mice;

10

Figure 5A and Figure 5B represent the ability of the IL5 monoclonal antibody to reduce the level of eosinophils in BALF.

DETAILED DESCRIPTION OF THE EMBODIMENTS

15 Terms

In order to understand the present disclosure more easily, certain technical and scientific terms are specifically defined below. All other technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which the present disclosure pertains, unless otherwise explicitly defined herein.

20

"Buffer" refers to a buffer that is resistant to changes in pH by the action of its acid-base conjugate components. Examples of the buffer which controls the pH within an appropriate range include acetate, succinate, gluconate, histidine salt, oxalate, lactate, phosphate, citrate, tartrate, fumarate, glycyl-glycine and other organic acid buffers.

25

"Histidine salt buffer" is a buffer comprising histidine ions. Examples of the histidine salt buffer include histidine-hydrochloride, histidine-acetate, histidine-phosphate, histidine-sulfate buffer, and the like; preferably, histidine-acetate buffer or histidine-hydrochloride buffer; histidine-acetate buffer is prepared by histidine and acetic acid, and histidine salt buffer is

prepared by histidine and HCl.

"Citrate buffer" is a buffer comprising citrate ions. Examples of the citrate buffer include citric acid-sodium citrate, citric acid-potassium citrate, citric acid-calcium citrate, citric acid-magnesium citrate, and the like. A preferred citrate buffer is citric acid-sodium citrate.

"Succinate buffer" is a buffer comprising succinate ions. Examples of the succinate buffer include succinic acid-sodium succinate, succinic acid-potassium succinate, succinic acid-calcium succinate, and the like. A preferred succinate buffer is succinic acid-sodium succinate.

"Phosphate buffer" is a buffer comprising phosphate ions. Examples of the phosphate buffer include disodium hydrogen phosphate-sodium dihydrogen phosphate, disodium hydrogen phosphate-potassium dihydrogen phosphate, disodium hydrogen phosphate- citric acid, and the like. A preferred phosphate buffer is disodium hydrogen phosphate-sodium dihydrogen phosphate.

"Acetate buffer" is a buffer comprising acetate ions. Examples of the acetate buffer include acetic acid-sodium acetate, histidine acetate, acetic acid-potassium acetate, acetic acid-calcium acetate, acetic acid-magnesium acetate, and the like. A preferred acetate buffer is acetic acid-sodium acetate.

"Pharmaceutical composition" means a mixture comprising one or more of the compounds (or physiological/pharmaceutically acceptable salt or prodrug thereof) described herein and other chemical components (such as physiological/pharmaceutically acceptable carriers and excipients). The purpose of a pharmaceutical composition is to maintain stability of antibody active ingredients, and to facilitate the administration to organism, so as to facilitate the absorption and the biological activity of the active ingredient.

As used herein, "pharmaceutical composition" and "formulation" are used interchangeably.

The "saccharide" in the present disclosure includes conventional $(\text{CH}_2\text{O})_n$ and the derivatives thereof, including monosaccharides, disaccharides, trisaccharides, polysaccharides, sugar alcohols, reducing saccharides, non-reducing saccharides, etc. The saccharide can be selected from the group consisting of: glucose, sucrose, trehalose, lactose, fructose, maltose, dextran,
 5 glycerol, erythritol, glycerol, arabitol, xylitol, sorbitol, mannitol, melibiose, melezitose, melitriose, mannotriose, stachyose, maltose, lactulose, maltulose, sorbitol, maltitol, lactitol, iso-maltulose, etc. The preferred saccharide is non-reducing disaccharide, more preferred sucrose.

10 According to the present disclosure, the solvent comprised in the solution form of the pharmaceutical composition is water, unless otherwise specified.

"Lyophilized formulation" means a formulation or a pharmaceutical composition obtained by vacuum lyophilization of the liquid or solution form of the pharmaceutical composition or the
 15 formulation.

The lyophilization in the present disclosure comprises pre-freezing, primary drying and secondary drying. The purpose of pre-freezing is to freeze the products to obtain crystalline solids. Pre-freezing temperature and pre-freezing speed are two important process parameters. In
 20 the present disclosure, the pre-freezing temperature is set to -45°C , with the pre-freezing speed at $1^\circ\text{C}/\text{min}$. The primary drying, also called main drying, is the main stage for lyophilization of samples. The purpose is to remove ice from the product while maintaining the shape of the product, so as to limit the damage to a product to minimum level. When the primary drying temperature and vacuum degree are not selected properly, the product would collapse. Higher
 25 temperature and higher vacuum degree accelerate the lyophilization efficiency, but also increase the risk of product collapse. The temperature of primary drying in present invention can be a conventional temperature in the field, such as -30°C to 0°C . The secondary drying is also called vacuum drying, is a main step which removes the bound water from a product by pumping an ultimate vacuum (0.01 mbar) and raising the temperature (20 to 40°C). Since most biological
 30 products are sensitive to temperature, the selected secondary drying temperature shall be at the lower point of the temperature range, which is 25°C . The time duration for lyophilization is

related to the freezer, the dose of the formulation to be lyophilized, and the container of the formulation to be lyophilized. Those skilled in the art well know how to adjust such time duration.

- 5 As used herein, the term "about" and "approximately" means that a value is within an acceptable error range of the specific value measured by an ordinary skilled in the art. The value partly depends on how the value is measured or determined (i.e. the limit of the measurement system). For example, in every practice in the art, "about" means a standard deviation within 1 or more than 1. As an alternative, "about" or "substantially comprising" means a range of at most $\pm 20\%$,
 10 for example, a pH of about 5.5 means $\text{pH } 5.5 \pm 1.1$. In addition, especially for biological systems or processes, the term means at most one order of magnitude or at most 5-folds of a value. Unless otherwise specified, when the specific value is indicated in the present application and claims, the meaning of "about" or "substantially comprising" should be within an acceptable error range of the specific value.

- 15 The pharmaceutical composition of the present disclosure is capable of achieving a stable effect: i.e., the antibody comprised in the pharmaceutical composition substantially retains the physical stability and/or chemical stability and/or biological activity following the storage. Preferably, the pharmaceutical composition substantially retains the physical stability and chemical stability as
 20 well as biological activity following the storage. The storage period is generally determined based on the predetermined shelf-life of the pharmaceutical composition. There are currently a number of analytical techniques for measuring the stability of a protein, which can be used to measure the stability after being stored for a selected period of time at a selected temperature.

- 25 A stable pharmaceutical formulation of antibody is a formulation for which no significant physical and/or chemical and/or biological change is observed under the following conditions: being stored at a cool temperature ($2-8^{\circ}\text{C}$) for at least 3 months, preferably 6 months, more preferably 1 year, and even more preferably up to 2 years. In addition, stable liquid formulations include those exhibit desirable characteristics after being stored at temperature of 25°C for 1
 30 month, 3 months or 6 months. Typically, criteria for stable formulation are as follows: typically no more than about 10%, preferably no more than about 5% of the antibody monomers being

degraded, as measured by SEC-HPLC; by visual inspection, the pharmaceutical formulation of antibody is light yellow, almost colorless clear liquid, or colorless, or clear to slightly pale; no more than $\pm 10\%$ variation occurs in the concentration, pH and osmolality of the formulation; generally no more than about 10%, preferably no more than about 5% of reduction is observed;
 5 generally no more than about 10%, preferably no more than about 5% of aggregation is formed.

An antibody would be deemed to "retain its physical stability" in the pharmaceutical formulation, when the antibody does not show a significantly increased aggregation, precipitation and/or denaturation, by visual inspection of color and/or clarity, or being measured via UV light
 10 scattering, size exclusion chromatography (SEC) and dynamic light scattering (DLS). Changes in protein conformation can be assessed by fluorescence spectroscopy, which determines the tertiary structure of a protein, and by FTIR spectroscopy, which determines the secondary structure of a protein.

15 An antibody would be deemed to "retain its chemical stability" in the pharmaceutical formulation, when the antibody does not show a significant chemical modification. Chemical stability can be assessed by detecting and quantifying chemically altered forms of a protein. Degradation processes that frequently alter the chemical structure of a protein include hydrolysis or truncation (assessed by methods such as size exclusion chromatography and SDS-PAGE), oxidation
 20 (assessed by methods such as peptide spectroscopy in combination with mass spectrometry or MALDI/TOF/MS), deamidation (assessed by methods such as ion exchange chromatography, capillary isoelectric focusing, peptide spectroscopy, isoaspartic acid measurement) and isomerization (assessed by methods such as measuring the content of isoaspartic acid, peptide spectroscopy).

25 An antibody would be deemed to "retain its biological activity" in the pharmaceutical formulation, when the biological activity of the antibody at a given time is still within the predetermined range of biological activity exhibited at the time when the pharmaceutical formulation was initially prepared. The biological activity of the antibody can be determined, for
 30 example, by antigen-binding assay.

The three-letter code and the one-letter code for amino acids used in the present disclosure are described in J. biol. chem. 243, p3558 (1968).

The "antibody" used in the present disclosure refers to immunoglobulin; a complete antibody is a tetra-peptide chain structure composed of two identical heavy chains and two identical light chains connected by inter-chain disulfide bond(s).

In the present disclosure, the antibody light chain of the present disclosure further comprises a light chain constant region, and the light chain constant region includes human or murine κ , λ chain or the variant(s) thereof.

In the present disclosure, the antibody heavy chain of the present disclosure further comprises a heavy chain constant region, and the heavy chain constant region includes human or murine IgG1, IgG2, IgG3, IgG4 or the variant(s) thereof.

The about 110 amino acid adjacent to the N-terminus of the antibody heavy and light chains are highly variable, known as variable regions (Fv regions); the rest of amino acid sequences close to the C-terminus are relatively stable, known as constant regions. The variable region includes three hypervariable regions (HVRs) and four relatively conservative framework regions (FRs).

The three hypervariable regions which determine the specificity of the antibody are also known as complementarity determining regions (CDRs). Each of the light chain variable region (LCVR, VL) and heavy chain variable region (HCVR, VH) consists of 3 CDR regions and 4 FR regions, with the sequential order from the amino terminus to carboxyl terminus of: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The three CDR regions of the light chain refer to LCDR1, LCDR2, and LCDR3, and the three CDR regions of the heavy chain refer to HCDR1, HCDR2, and HCDR3. The number and position of the CDR amino acid residues of the LCVR region and the HCVR region in the antibody or the antigen-binding fragment thereof described in the present disclosure comply with the known Kabat numbering criteria (LCDR 1 to 3, HCDR 1 to 3).

The antibodies of the present disclosure include murine antibodies, chimeric antibodies, humanized antibodies, and preferably humanized antibodies.

- 5 In the present disclosure, the "antigen-binding fragment of an antibody" or "functional fragment" refers to Fab fragments, Fab' fragments, F(ab')₂ fragments that have antigen-binding activity, and Fv fragments, scFv fragments that bind to antibody. Fv fragment comprises a heavy chain variable region and a light chain variable region of the antibody, but does not have a constant region, and Fv fragment is the smallest antibody fragment that has all antigen binding sites.
- 10 Generally, the Fv antibody also comprises a polypeptide linker between VH and VL domains, and is capable of forming a structure required for antigen-binding. Different linkers can also be used to connect two antibody variable regions to form a polypeptide chain, named single chain antibody or single chain Fv (sFv).
- 15 The term "antigen-binding site" of the present disclosure refers to a continuous or discontinuous three-dimensional spatial site on an antigen recognized by the antibody or antigen-binding fragment thereof of the present disclosure.

The term "murine antibody" in the present disclosure refers to a monoclonal antibody against
 20 human IL-5 prepared according to the knowledge and skills in the art. During the preparation, the test subject is injected with IL-5 antigen, and then hybridoma expressing antibody having desired sequence or functional feature is isolated.

The term "chimeric antibody" is an antibody formed by fusing the variable region of a murine
 25 antibody with the constant region of a human antibody, which reduces the immune response induced by the murine antibody. To establish a chimeric antibody, it is necessary to firstly establish a hybridoma secreting murine specific monoclonal antibodies; then the variable region

genes are cloned from the mouse hybridoma cells; and then the constant region genes of the human antibodies are cloned as needed; and the murine variable region genes are combined with the human constant region genes to form a chimeric gene which is inserted into a human vector, and finally the chimeric antibody molecule is expressed in a eukaryotic industrial system or a prokaryotic industrial system. In a preferred embodiment of the present disclosure, the light chain of IL-5 chimeric antibody further comprises the light chain constant region of human κ , λ chain or the variant(s) thereof. The heavy chain of the IL-5 chimeric antibody further comprises the heavy chain constant region of human IgG1, IgG2, IgG3, IgG4 or the variant(s) thereof. The constant region of human antibody can be selected from the group consisting of: the heavy chain constant region of human IgG1, IgG2, IgG3 or IgG4 or the variant(s), preferably comprises human IgG2 or IgG4 heavy chain constant region, or IgG4 without ADCC toxicity (antibody-dependent cell-mediated cytotoxicity) after amino acid mutation.

The term "humanized antibody", also known as CDR-grafted antibody, refers to an antibody generated by grafting the murine CDR sequences onto human antibody variable region framework, i.e., an antibody produced in different types of human germline antibody framework sequences. The humanized antibody avoids strong heterogeneous responses induced by chimeric antibody which carries a large number of murine protein components. Such framework sequences can be obtained from public DNA database covering germline antibody gene sequences or published references. For example, germline DNA sequences of human heavy and light chain variable region genes can be found in "VBase" human germline sequence database (available on www.mrccpe.com.ac.uk/vbase), as well as in Kabat, EA, et al. 1991 Sequences of Proteins of Immunological Interest, 5th Ed. To avoid a decrease in activity caused by the decreased immunogenicity, the framework sequences in human antibody variable region may be subjected to minimal reverse-mutation or back-mutation so as to maintain the activity. The humanized antibody of the present disclosure also refers to a humanized antibody on which CDRs affinity maturation is performed by phage display.

In the present disclosure, the "ADCC" (i.e. antibody-dependent cell-mediated cytotoxicity) means that antibody-coated target cells are directly killed by cells expressing Fc receptors, through recognizing the Fc segment of antibody. The ADCC effector function of the antibody
 5 can be reduced or eliminated by modifying the Fc segment of IgG. The modification refers to mutations performed on the heavy chain constant region of an antibody, such as selected from the group consisting of: N297A, L234A, L235A on IgG1; IgG2/4 chimera, F234A/L235A mutation on IgG4.

10 The "mutation" in a mutant sequence in the present disclosure includes but is not limited to "back-mutation(s)", "conservative modification" or "conservative replacement or substitution". The "conservative modification" or "conservative replacement or substitution" used in the present disclosure refers to other amino acids with similar characteristics (such as charge, side chain size, hydrophobicity/hydrophilicity, backbone conformation and rigidity, etc.) are used to
 15 replace the amino acids in a protein, so that replacement can be frequently performed without changing the biological activity of a protein. Those skilled persons in the art know that, generally, single amino acid substitution in a non-essential region of a polypeptide does not substantially change the biological activity (see, for example, Watson et al., (1987) Molecular Biology of the Gene, The Benjamin/Cummings Pub. Co., Page 224, (the 4th edition)). In addition, the
 20 substitution of amino acids with similar structure or function is unlikely to disrupt the biological activity.

The "mutated sequence" in the present disclosure refers to that the nucleotide sequence and/or amino acid sequence of the present disclosure are appropriately modified by mutation(s) (such as
 25 substitution, insertion or deletion) to obtain a nucleotide sequence and/or amino acid sequence which has different sequence identity percentage with the nucleotide sequence and/or amino acid sequence of the present disclosure. In the present disclosure, the sequence identity can be at least

85%, 90% or 95%, preferably at least 95%. Non-limiting examples refer to 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. Sequence alignment and determination of identity percentage between two sequences can be performed by the default settings of BLASTN/BLASTP algorithm available on the website of National Center
 5 For Biotechnology Institute.

The term "binding to IL-5" refers to an interaction with IL-5, preferably with human IL-5.

The terms "anti-IL-5 antibody" and "IL-5 antibody" are used interchangeably, and both refer to
 10 antibody that binds to IL-5.

In the present disclosure, the fusion protein is a protein product obtained by co-expressing two genes through DNA recombination. The recombinant IL-5 extracellular region-Fc fusion protein is a fusion protein obtained by co-expressing IL-5 extracellular region and human antibody Fc
 15 fragment, through DNA recombination. The IL-5 extracellular region refers to the portion of IL-5 protein expressed outside the cell membrane, and the sequence thereof is as shown in SEQ ID NO: 1.

The methods for producing and purifying antibodies and antigen-binding fragments are well
 20 known and available in the prior art, such as Antibodies: A Laboratory Manual, Cold Spring Harbor press, Chapters 5-8 and 15. For example, mice can be immunized with a human IL-5 or fragment thereof, and the resulting antibody can be renatured and purified, and amino acid sequencing can be performed by using conventional methods. Antigen-binding fragments can also be prepared by using conventional methods. The antibody or antigen-binding fragment
 25 according to the present disclosure is genetically engineered to graft one or more human FR region(s) onto the non-human CDR regions. The human FR germline sequences can be obtained from ImMunoGeneTics (IMGT) website <http://imgt.cines.fr>, or can be obtained from The

Immunoglobulin Journal, 2001 ISBN 012441351.

The engineered antibody or antigen-binding fragment thereof can be prepared and purified by conventional methods. For example, the cDNA sequences encoding the heavy chain and light chain can be cloned and recombined into a GS expression vector. The expression vectors of recombinant immunoglobulin can be stably transfected into CHO cells. As a recommended prior art, mammalian expression systems can lead to glycosylation of antibodies, especially at highly conservative N-terminal positions of the Fc region. Stable clones are obtained by expressing antibodies that specifically bind to human IL-5. Positive clones are expanded in serum-free culture medium of bioreactors to produce antibodies. The culture medium into which the antibodies are secreted can be purified by conventional techniques. For example, Protein A or Protein G Sepharose FF column comprising adjusted buffer can be used for purification. Non-specifically bound components are washed out. Then the bound antibodies are eluted by pH gradient, and the antibody fragments are detected by SDS-PAGE and collected. The antibodies can be filtered and concentrated by conventional methods. Soluble mixtures and multimers can also be removed by conventional methods, for example molecular sieves and ion exchange. The resulting product shall be frozen immediately, such as at -70°C, or lyophilized.

"Optional" or "optionally" means that the event or environment that follows the term can but does not have to occur, and the description involves occasions where the event or environment would occur or not occur. For example, "optionally comprises 1 to 3 heavy chain CDR region(s) of an antibody" means that the heavy chain CDR region(s) of an antibody having specific sequence(s) can, but not necessarily, be present.

"Administering" and "treating", when applied to animals, humans, experimental subjects, cells, tissues, organs or biological fluids, refer to a contact of exogenous medicament, therapeutic agent, diagnostic agent or composition with the animals, humans, subjects, cells, tissues, organs

or biological fluids. "Administering" and "treating" can refer to for example treatment, pharmacokinetics, diagnosis, research and experimental methods. The treatment of cells includes a contact of reagent with cells, and a contact of reagent with fluids, wherein the fluids are in contact with the cells. "Administering" and "treating" for example also mean treatment of cells
 5 by reagents, diagnostic agent, binding compositions or by another cell *in vitro* and *ex vivo*. "Treating" when applied to human, veterinary or research subjects, refers to therapeutic treatment, prevention or prophylactic measures, research and diagnostic applications.

"Treatment" means applying an internal or external therapeutic agent, for example a composition
 10 comprising any one of the binding compounds of the present disclosure, to a patient who has one or more disease symptoms on which the therapeutic agent is known to have therapeutic effect. Generally, the therapeutic agent is given at an amount effective to alleviate one or more disease symptoms in the treated patient or population to induce the regression of such symptoms or inhibit the development of such symptoms to any clinically detectable extent. The amount of
 15 therapeutic agent that is effective to alleviate any specific disease symptom (also referred to as a "therapeutically effective amount") can vary according to a variety of factors, for example the patient disease state, age and body weight, and the ability of the agent to produce the desired therapeutic effect in the patient. Whether the disease symptoms have been alleviated can be evaluated through any clinical testing methods commonly used by doctors or other health care
 20 professionals to evaluate the severity or progression of the symptoms. Although an embodiment of the present disclosure (for example treatment method or article of manufacture) may not be effective in alleviating each target disease symptom, it should alleviate the target disease symptom in a statistically significant number of patients, as determined according to any statistical test methods known in the art, such as Student t-test, chi-square test, Mann and
 25 Whitney's U test, Kruskal-Wallis test (H test), Jonckheere-Terpstra test and Wilcoxon test.

There is no restriction to diseases related to IL-5, as long as it is a disease related to IL-5. For example, the therapeutic response induced by the molecule of the present disclosure can be produced by binding to human IL-5 and subsequently blocking or inhibiting the stimulation effects of eosinophils. Therefore, when applied to preparations and formulations suitable for therapeutic application, the pharmaceutical composition of the present disclosure is very useful for such subjects who suffer from allergies and/or atopic reactions, or suffer from reactions related to eosinophils, such as but not limited to asthma, exacerbation of asthma, malignant onset of asthma, chronic pneumonia, allergic rhinitis, perennial allergic rhinitis, allergic bronchopulmonary aspergillosis disease, eosinophilia, Churg-Strauss syndrome, atopic dermatitis, onchocerciasis dermatitis, intermittent angioedema, eosinophilic myalgia syndrome, eosinophilic gastroenteritis, worm infection, Hodgkin's disease, nasal polyps, Loeffler's syndrome, urticaria, hypereosinophilic bronchitis, nodular arteritis, sinusitis, eosinophilic esophagitis, allergic eosinophilic esophagitis, allergic conjunctivitis, onchocerciasis dermatitis, endometriosis, or steroid-dependent eosinophilic bronchitis, etc. In a preferred embodiment, the treatment can inhibit or alleviate the infiltration of eosinophils into lung tissue. The frequency of administration for the pharmaceutical composition can be from three times per day to once every 6 months. The route of administration can be intravenous, subcutaneous, intramuscular, parenteral or topical route.

The formulations of the present disclosure can be used to treat IL-5 mediated diseases. For example, the formulations can be used to, but not limited to, inhibit or alleviate IL-5 mediated inflammatory response and the maturation, activation, degranulation or tissue infiltration of eosinophils *in vivo* and *in vitro*; inhibit the excessive stress of smooth muscle caused by IL-5; reduce the level of IL-5 in lung, airway or blood. Preferably, the formulation of the present disclosure can be used to treat IL-5 mediated diseases, preferably, the IL-5 mediated disease is selected from the group consisting of: asthma, chronic pneumonia, allergic rhinitis, allergic bronchopulmonary aspergillosis disease, eosinophilia, Churg-Strauss syndrome, atopic dermatitis,

onchocerciasis dermatitis, intermittent angioedema, eosinophilic myalgia syndrome, eosinophilic gastroenteritis, worm infection, Hodgkin's disease, nasal polyps, Loeffler's syndrome, urticaria, hypereosinophilic bronchitis, nodular arteritis, sinusitis, eosinophilic esophagitis, allergic eosinophilic esophagitis, allergic conjunctivitis, onchocerciasis dermatitis, endometriosis and
 5 steroid-dependent eosinophilic bronchitis.

"Effective amount" includes an amount sufficient to improve or prevent the symptoms or conditions of medical disease. An effective amount also refers to an amount sufficient to allow or facilitate diagnosis. The effective amount for a particular patient or veterinary subject may vary
 10 depending on the following factors: for example, the condition to be treated, the patient's general condition, the route of administration and dosage, and the severity of side effects. The effective amount can be a maximum dose or dosing schedule that avoids significant side effects or toxic effects.

15 "Exchange" refers to the exchange of the solvent system that is used to dissolve the antibody protein. For example, a high salt or hypertonic solvent system comprising an antibody protein is replaced with a buffer system of a stable formulation, by physical manipulation, so that the antibody protein would be present in a stable formulation. The physical manipulation includes but not limited to ultrafiltration, dialysis or reconstitution after centrifugation.

20

Examples and Test Examples

The present disclosure is further described in conjunction with the examples below, but these examples do not limit the scope of the present disclosure. The experimental methods that are not provided with specific conditions in the example of the present disclosure are usually in
 25 accordance with conventional conditions, such as reference to the publications of Antibodies: A Laboratory Manual, Molecular Cloning by Cold Spring Harbor Laboratory; or in accordance with the conditions recommended by the manufacturers of materials or products. Reagents that

are not provided with specific sources are conventional reagents purchased on the market.

Example 1. Preparation of IL-5 antigen and protein used in the detection

1.1 Design and expression of IL-5 antigen

5 The sequences encoding human IL-5 with His-tag, rhesus IL-5 with His-tag, mouse IL-5 with His-tag, rat IL-5 with His-tag, human IL-5R α receptor extracellular region fusion protein comprising human IgG1-Fc fragment were cloned into pHR vectors, and expression plasmids were constructed and then transfected into HEK293. On day 6 after transfection, samples were collected, and the cell supernatant was collected by centrifugation at 4500 rpm for 10 min. The
10 supernatants comprising the recombinant IL-5 and IL-5 α receptor proteins were purified by using nickel column, the recombinant human IL-5-Fc fusion protein was purified by using Protein A affinity chromatography column. The purified protein can be used in subsequent experiments. The sequences for the specific protein antigens are as follows:

15 The amino acid sequence for human IL-5 with his-tag (rhIL-5-his)
MRMLLHLSLLALGAAYVYAIPTEIPTSA~~LVKETLALLSTHRTLLIANETLRIPVPVHKNHQ~~
LCTEEIFQGIGTLESQTVQGGTVERL~~FKNLSLIKKYIDGQKKKCGEERRRVNQFLDYLQE~~
FLGVMNTEWIIESHHHHHH

Note: The portion in italics represents His6-tag;

20 SEQ ID NO: 1

The amino acid sequence for cyno IL-5 with his-tag
MRMLLHLSLLALGAAYVYAIPTEIPASALVKETLALLSTHRTLLIANETLRIPVPVHKNHQ
LCTEEIFQGIGTLESQTVQGGTVERL~~FKNLSLIKKYIGGQKKKCGEERRRVNQFLDYLQE~~
FLGVMNTEWIIESHHHHHH

25 SEQ ID NO: 2

Note: The portion in italics represents His6-tag.

The amino acid sequence for mouse IL-5 with his-tag

MEIPMSTVVKETLTQLSAHRALLTSNETMRLPVPTHKNHQLCIGEIFQGLDILKNQTVRG
 GTVEMLFQNLSLIKKYIDRQKEKCGEERRRTRQFLDYLQEFLGVMSTEWAMEGHHHHH
H

5 SEQ ID NO: 3

Note: The portion in italics represents His6-tag.

The amino acid sequence for rat IL-5 with his-tag

MEIPMSTVVKETLIQLSTHRALLTSNETMRLPVPTHKNHQLCIGEIFQGLDILKNQTVRG
 10 GTVEILFQNLSLIKKYIDGQKEKCGEERRKTRHFLDYLQEFLGVMSTEWAMEVHHHHH
H

SEQ ID NO: 4

Note: The portion in italics represents His6-tag.

15 The amino acid sequence for human IL-5 α receptor fused to human Fc fragment

DLLPDEKISLLPPVNFTIKVTGLAQVLLQWKPNPDQEQRVNLEYQVKINAPKEDDYET
RITESKCVTILHKGFSASVRTILQNDHSLASSWASAELHAPPGSPGTSIVNLTCTNTTED
NYSRLRSYQVSLHCTWLVGTDAPEDTQYFLYYRYGSWTEECQEYSKDTLGRNIACWFP
RTFILSKGRDWLAVLVNGSSKHSAIRPFDQLFALHAIDQINPPLNVTAEIEGTRLSIQWEKP
 20 VSAFPIHCFDYEYVKIHNTNRNGYLQIEKLMTNAFISIIDDLSKYDVQVRAAVSSMCREAGL
WSEWSQPIYVGNDDEHKPLREWIEGRMDEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
 25 *YTQKSLSLSPGK*

SEQ ID NO: 5

Note: The portion in italics represents human IgG1-Fc-tag.

Example 2. Construction and identification of recombinant IL-5 α receptor and IL-5 α / β receptor cell lines

To screen for functional antibodies, the present disclosure constructed CHO-S/IL-5 α cell lines
 5 expressing IL-5 α , and CHO-S/IL-5 α /IL-5 β cell lines expressing both IL-5 α and IL-5 β .

Specifically, the human IL-5 α full-length gene (Q01344) was cloned into a mammalian cell
 expression vector pTargetT, the linearized plasmid was electro-transfected into CHO-S cells, and
 screened in the presence of G418 for 2 weeks, and then limited dilutions were performed twice.

10 The IL-5 α gene was detected on cell surface by FACS, and the CHO-S/IL-5 α cell line with high
 IL-5 α expression level was selected. On this basis, the linearized pcDNA3.1-IL-5 β was
 electro-transfected, and screened in the presence of G418 and zeocin for 2 weeks, and then
 limited dilutions were performed twice. The IL-5 α and IL-5 β genes were detected on the cell
 surface by FACS, and CHO-S/IL-5 α /IL-5 β cell line with high expression level of IL-5 α and
 15 IL-5 β was selected.

Example 3. Preparation of anti-human IL-5 murine monoclonal antibody

Two doses of the recombinant protein rhIL-5-his, Freund's adjuvant CFA (Sigma, Lot#
 SLBQ1109V), and IFA (Sigma, Lot#SLBJ2845V) (100g/50g/50g (high) and 25g/12.5g/12.5g
 20 (low)) were used to immunize two groups of Balb/c mice (5 mice/group) and four groups of SJL
 mice (5 mice/group), respectively. The specific immune response to IL-5 was determined by
 detecting serum titer by ELISA, by ligand-receptor blocking assay and by inhibition assay of
 TF-1 proliferation. The mice with good specific immune response were selected and sacrificed;
 the spleen cells were collected and fused with myeloma cells.

25 The primary screening was carried out by ELISA binding assay against human IL-5. Once the
 hybridoma cells were transferred to a 24-well plate, the supernatants were screened again, by

using ELISA binding assay against human, cynomolgus monkey and mouse IL-5, ELISA-based receptor blocking assay against IL-5, and inhibition assay of TF-1 proliferation. After the positive clones were subjected to two rounds of sub-cloning, the hybridoma clones were obtained and were used for antibody production; and the obtained antibodies were purified by affinity chromatography.

The purified antibodies were subjected to: SEC-HPLC, detection of endotoxin content, Biacore affinity assay for various species IL-5, FACS-based receptor blocking assay against IL-5, inhibition assay of TF-1 proliferation, adhesion test of eosinophils, and efficacy evaluation in mouse model of asthma and neutralization model of guinea pig *in vivo*; the monoclonal hybridoma cell lines mAb1705, mAb1706, mAb1780, mAb1773 and mAb1779 showing favorable activity *in vivo* and *in vitro* were selected.

The process for cloning sequences from positive hybridoma was as follows: hybridoma cells at logarithmic growth phase were collected, RNA was extracted with Trizol (Invitrogen, Cat No.15596-018) according to the kit instructions, PrimeScript™ Reverse Transcriptase Kit was used for reverse transcription (Takara, Cat No. 2680A). The cDNA obtained by reverse transcription was amplified by PCR using mouse Ig-Primer Set (Novagen, TB326 Rev. B0503) and then sequenced. The amino acid sequences corresponding to the heavy chain and the light chain variable region DNA sequences for mAb1705, mAb1706, mAb1780, mAb1773 and mAb1779 were obtained (the amino acid residues of VH/VL CDRs are determined and annotated by Kabat numbering criteria).

The sequence for mAb1705 murine heavy chain variable region

EVQLVESGGGLVQPGRSLKLSCTASGFTFSHYYMAWVRQAPKKGLEWVTSISYEGDITY
YGDSVKGRFTISRDNASTLYLQMNSLRSEDTATYYCASQTLRESFDYWGQGVMVTVS
S

SEQ ID NO: 6

The sequence for mAb1705 murine light chain variable region

DIQMTQSPSSMSVSLGDRVITITCRASQDIANYLSWYQQKIARSPKLVIIYGTSNLEVGVP

5 RFSGSRSGSDYSLTINTLESEDTGIYFCLQDKEFPRTFGGGTRLELK

SEQ ID NO: 7

The sequence for mAb1706 murine heavy chain variable region

EVQLVESGGGLVQPGRSLKLSAASGFTFSHYYMAWVRQAPKKGLEWVTSINYEGNSA

10 YYGDSVKGRFTISRDNASTLYLQMDSLRSEDTATYYCATETLRESLDYWGQGVMTV
SS

SEQ ID NO: 8

The sequence for mAb1706 murine light chain variable region

15 DIQMTQSPSSMSVSLGDRVITITCRASQDIGNYLSWYQQKLGKSPKLMIHSASNLEVGVP
RFSGSRSGSDYSLTINTLESEDPGIYFCLQHKQFPRTFGGGTKLELK

SEQ ID NO: 9

The sequence for mAb1780 murine heavy chain variable region

20 QVKLLQSGAALVKPGDSVKMSCKASDYTFTEYLIHWVKQSQGRSLEWIGYINPYSGGT
VYNEKFKSKATLTVDKFSSTAYMEFRRLTFEDSAIYYCARDGGYSDPLDYWGQGVMT
VSS

SEQ ID NO: 10

25 The sequence for mAb1780 murine light chain variable region

DTVLTQSPALAVSPGERVSISCRASEGLTSYMHWYQQKPGQPKLLIYKASNLASGVPAR
FSGSGSGTDFTLTIDPVEADDAATYFCQQNWNDPWTFGGGKLELK

SEQ ID NO: 11

The sequence for mAb1773 murine heavy chain variable region

EVQLQQSLAELVRPGASVTLSTASGFNIKNTYIHWVKQRPEQGLEWIGRIDPANGDTKH

5 GPKFQGKATITADTSSNTAYLQFSSLTSEDTAIYYCFRYGIYPDHWGQGTPLTVSS

SEQ ID NO: 12

The sequence for mAb1773 murine light chain variable region

QIVLTQSPALMSASPGEKVTMTCSSASSSVNYIYWYQQKPRSSPKPWIYLTATLASGVPAR

10 FSGSGSGTSFSLTISRMEAEDAATYYCQQWNSYPYTFGGGGTKLEIE

SEQ ID NO: 13

The sequence for mAb1779 murine heavy chain variable region

QVKLLQSGAALVKPGDSVKMSCKASGYTFTDYIIHWVKQSHGKSLEWIGYFNPNSGGS

15 NYNENFKRKATLTADKSSSTAYLEFSRVTSEDSAIYYCGRRIAWDHWYFDFWGPGTMTMT
VSS

SEQ ID NO: 14

The sequence for mAb1779 murine light chain variable region

20 DIQMTQSPASLSASLGETVSIECLASEGISNDVAWYQQKSGRSPQLLVYAASRLQDGVPS

RFSGSGSGTRYFFKISGMQPEDEADYFCQQGYKTPLTFGSGGTKLEIK

SEQ ID NO: 15.

The CDR sequences of light and heavy chain for each antibody are shown in Table 1.

25

Table 1. The sequences for CDR regions in heavy chain and light chain of each antibody

Antibody	heavy chain		light chain	
mAb1705	HCDR1	HYHYMA SEQ ID NO: 16	LCDR1	RASQDIANYLS SEQ ID NO: 19
	HCDR2	SISYEGDITYYGDSVKG SEQ ID NO: 17	LCDR2	GTSNLEV SEQ ID NO: 20
	HCDR3	QTLRESFDY SEQ ID NO: 18	LCDR3	LQDKEFPRT SEQ ID NO: 21
mAb1706	HCDR1	HYHYMA SEQ ID NO: 22	LCDR1	RASQDIGNYLS SEQ ID NO: 25
	HCDR2	SINYEGNSAYYGDSVKG SEQ ID NO: 23	LCDR2	SASNLEV SEQ ID NO: 26
	HCDR3	ETLRESLDY SEQ ID NO: 24	LCDR3	LQHKQFPRT SEQ ID NO: 27
mAb1780	HCDR1	EYLIH SEQ ID NO: 28	LCDR1	RASEGLTSYMH SEQ ID NO: 31
	HCDR2	YINPYSGGTVYNEKFKS SEQ ID NO: 29	LCDR2	KASNLAS SEQ ID NO: 32
	HCDR3	DGGYSDPLDY SEQ ID NO: 30	LCDR3	QQNWNDPWT SEQ ID NO: 33
mAb1773	HCDR1	NTYIH SEQ ID NO: 34	LCDR1	SASSSVNYIY SEQ ID NO: 37
	HCDR2	RIDPANGDTKHGPKFQG SEQ ID NO: 35	LCDR2	LTATLAS SEQ ID NO: 38
	HCDR3	YGIYPDH SEQ ID NO: 36	LCDR3	QQWNSYPYT SEQ ID NO: 39
mAb1779	HCDR1	DYIIH SEQ ID NO: 40	LCDR1	LASEGISNDVA SEQ ID NO: 43
	HCDR2	YFNPNSSGGSNYNENFKR SEQ ID NO: 41	LCDR2	AASRLQD SEQ ID NO: 44
	HCDR3	RIAWDHWYFDF SEQ ID NO: 42	LCDR3	QQGYKTPLT SEQ ID NO: 45

The detection result of activity by Biacore can be found in Table 2.

Table 2. *In vitro* activity of IL-5 murine antibody

Antibody	HuIL-5 affinity (KD (M))
mAb1705	7.27E-11
mAb1706	3.83E-11
mAb1780	8.99E-11
mAb1773	1.29E-10
mAb1779	4.58E-10

The results show that the murine antibodies of the present disclosure have high affinity for the antigen.

Example 4. Purification of IL-5 related recombinant proteins, and purification of hybridoma antibodies and recombinant antibodies

4.1 Purification steps of IL-5-Flag-His recombinant protein:

The samples were centrifuged at high speed to remove impurities and concentrated to an appropriate volume. The NI-NTA affinity column (QIAGEN, Cat No. 30721) was equilibrated with PBS, and washed with 2-5 folds of column volume. After removing impurities, the cell expression supernatant sample was loaded onto the column. The column was washed with PBS, until the A280 reading was decreased to baseline. The column was washed with PBS to remove the contaminating proteins, the sample was collected. The target protein was eluted successively with washing buffer (20mM imidazole) and elution buffer (300mM imidazole), and the elution peaks were collected.

The collected eluate was further purified by ion exchange (Hiload 16/600 superdex 200 column). The column was equilibrated with about 2-column volumes of PBS to ensure pH at 7.4. The elution buffer comprising the identified target protein was concentrated and loaded, the sample was collected and verified by SDS-PAGE and LC-MS identification, and aliquoted for use.

4.2 Purification of hybridoma expressed antibody and Fc fusion protein

The cell expression supernatant sample was centrifuged at high speed to remove impurities. The hybridoma expression supernatant was purified with Protein G column, and the Fc fusion protein expression supernatant was purified with Protein A column. The column was washed with PBS until the A280 reading was decreased to baseline. The target protein was eluted with 100mM acetic acid pH 3.0, and neutralized with 1M Tris-HCl, pH 8.0. After the eluted sample was properly concentrated, the sample was further purified by PBS-equilibrated gel chromatography

Superdex200 (GE), and the peak without aggregates was collected and then aliquoted for use.

Example 5. Humanization design of anti-human IL-5 monoclonal antibody

The humanization of the murine anti-human IL-5 monoclonal antibody was carried out as disclosed in many references in the art. In brief, the constant regions of the murine antibody were replaced with human constant regions, and the CDRs of the murine antibody were grafted onto FR human template having the highest homology, and back-mutation was performed on the amino acids in the FR region.

By aligning against the IMGT human antibody heavy and light chain variable region germline gene database, the heavy chain and the light chain variable region germlines that have high identity with each of amino acid sequences of mAb-1705, mAb-1706, mAb1780, mAb1773 and mAb1779 antibodies were selected as templates, the CDRs of the murine antibody were grafted onto the corresponding human template to form a variable region in the order of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. The amino acid residues were determined and annotated by Kabat numbering criteria.

The selection of human FR regions and amino acid back-mutation

On the basis of typical structure of the obtained murine antibody VH/VL CDR, the light chain variable region (VL) and the heavy chain variable region (VH) homologous sequences were retrieved from human germline database, and ranked according to FR homology (from high to low); the germline with the highest FR homology was selected as main template. The CDR regions of the murine antibody were grafted onto the human template, and then the FR residues were subjected to mutation, and the amino acid residues were optimized to obtained the final humanized molecules.

5.1 The selection of humanized framework for hybridoma clone mAb1705

For h1705, IGHV3-23*04 was selected as the template for VH, and IGKV1-12*01 was selected as the template for VL. The CDRs of mAb1705 were grafted onto the human template; the embedded residues and residues which directly interacted with CDR region (found through software) were subjected to back-mutation. Various light chain and heavy chain variable regions of the humanized antibodies were obtained, as shown in Table 3.

Table 3. Template selection and back-mutation design for h1705

h1705_VL		h1705_VH	
h1705_VL.1	Grafted	h1705_VH.1	Grafted
h1705_VL.1A	A43S, G66R	h1705_VH.1A	K98S
h1705_VL.1B	A43S, L47V, G66R, T69S, F71Y, Y87F	h1705_VH.1B	S49T, V93T, K98S

Note: “Grafted” represents that the murine antibody CDRs were grafted onto the human germline FR region sequence. For example, according to the natural numbering of amino acid sequence, A43S represents that A at position 43 of “grafted” was mutated back to S.

Table 4. Combination of h1705 humanized antibody heavy and light chain variable regions

	h1705_VH.1	h1705_VH.1A	h1705_VH.1B
h1705_VL.1	h1705-003	h1705-004	h1705-005
h1705_VL.1A	h1705-006	h1705-007	h1705-008
h1705_VL.1B	h1705-009	h1705-010	h1705-011

Note: This table shows the sequences obtained by various combinations of mutations. As indicated by h1705-007, the humanized murine antibody h1705-007 comprises two mutants, i.e., light chain h1705_VL.1A and heavy chain h1705_VH.1A, and so forth.

The specific sequences of the variable regions of the humanized antibody h1705 are as follows:

> h1705_VL.1 (SEQ ID NO: 46)

DIQMTQSPSSVSASVGDRVITTCRASQDIANYLSWYQQKPGKAPKLLIYGTSNLEVGVP
RFSGSGSGTDFTLTISLQPEDFATYYCLQDKEFPRTFGGGTKVEIK

5 > h1705_VL.1A (SEQ ID NO: 47)

DIQMTQSPSSVSASVGDRVITTCRASQDIANYLSWYQQKPGKSPKLLIYGTSNLEVGVP
RFSGSRSGTDFTLTISLQPEDFATYYCLQDKEFPRTFGGGTKVEIK

> h1705_VL.1B (SEQ ID NO: 48)

10 DIQMTQSPSSVSASVGDRVITTCRASQDIANYLSWYQQKPGKSPKLVIYGTSNLEVGVP
RFSGSRSGSDYTLTISLQPEDFATYFCLQDKEFPRTFGGGTKVEIK

> h1705_VH.1 (SEQ ID NO: 49)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVSSISYEGDITY
15 YGDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKQTLRESFDYWGQGLTVTVSS

> h1705_VH.1A (SEQ ID NO: 50)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVSSISYEGDITY
YGDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCASQTLRESFDYWGQGLTVTVSS

20

> h1705_VH.1B (SEQ ID NO: 51)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVTSISYEGDITY
YGDSVKGRFTISRDN SKNTLYLQMNSLRAEDTATYYCASQTLRESFDYWGQGLTVTVSS.

25 Each of the light chain variable regions as described above was combined with the light chain constant region to form a light chain sequence, and each of the heavy chain variable regions was combined with the heavy chain constant region to form a heavy chain sequence. The exemplary humanized antibody constant region sequences are shown below:

> heavy chain IgG1 constant region:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
 5 PSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
 DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
 RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 52

10 Note: the underlined portion represents M252Y, S254T, T256E mutations.

> light chain kappa constant region:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

15 SEQ ID NO: 53.

5.2 The selection of humanized framework for hybridoma clone mAb1706

For h1706, IGHV3-23*04 was selected as the template for VH, and IGKV1-12*01 was selected
 as the template for VL. The CDRs of murine antibody mAb1706 were grafted onto the selected
 20 humanized template; the FR amino acids were subjected to back-mutation. The light chain and
 heavy chain variable regions of the humanized antibodies were obtained, as shown in Table 5.

Table 5. Template selection and back-mutation design for h1706

h1706_VL		h1706_VH	
h1706_VL.1	Grafted	h1706_VH.1	Grafted
h1706_VL.1A	A43S	h1706_VH.1A	K98T
h1706_VL.1B	A43S, L47M, F71Y, Y87F	h1706_VH.1B	S49T, V93T, K98T

Note: “Grafted” represents that the murine antibody CDRs were grafted onto the human
 25 germline FR region. For example, according to the natural numbering of amino acid sequence,

A43S represents that A at position 43 of “grafted” was mutated back to S.

The designed humanized molecules were combined to form different antibodies shown in the following table, as shown in Table 6.

5

Table 6. Combination of h1706 humanized antibody heavy and light chain variable regions

	h1706 VH.1	h1706 VH.1A	h1706 VH.1B
h1706 VL.1	h1706-002	h1706-003	h1706-004
h1706 VL.1A	h1706-005	h1706-006	h1706-007
h1706 VL.1B	h1706-008	h1706-009	h1706-010

The specific sequences of the variable regions of the h1706 humanized antibody are as follows:

10 > h1706_VL.1 (SEQ ID NO: 54)

DIQMTQSPSSVSASVGDRVITTCRASQDIGNYLSWYQQKPGKAPKLLIYSASNLEVGVP
RFSGSGSGTDFTLTISLQPEDFATYYCLQHKQFPRTFGGGTKVEIK

> h1706_VL.1A (SEQ ID NO: 55)

15 DIQMTQSPSSVSASVGDRVITTCRASQDIGNYLSWYQQKPGKSPKLLIYSASNLEVGVP
RFSGSGSGTDFTLTISLQPEDFATYYCLQHKQFPRTFGGGTKVEIK

> h1706_VL.1B (SEQ ID NO: 56)

DIQMTQSPSSVSASVGDRVITTCRASQDIGNYLSWYQQKPGKSPKLLIYSASNLEVGVP
20 RFSGSGSGTDYTLTISLQPEDFATYFCLQHKQFPRTFGGGTKVEIK

> h1706_VH.1 (SEQ ID NO: 57)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVSSINYE
YGD
25 SS

> h1706_VH.1A (SEQ ID NO: 58)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVSSIN YEGNSA
YYGDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATETLRESLDYWGQGMVTV
SS

5

> h1706_VH.1B (SEQ ID NO: 59)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVTSIN YEGNSA
YYGDSVKGRFTISRDN SKNTLYLQMNSLRAEDTATYYCATETLRESLDYWGQGMVTV
SS.

10

Each of the light chain variable regions as described above was combined with the light chain constant region to form a light chain sequence. Each of the heavy chain variable regions was combined with the heavy chain constant region to form a heavy chain sequence.

15 5.3 The selection of humanized framework for hybridoma clone mAb1780

For h1780, IGHV1-2*02 was selected as the template for VH, and IGKV3-11*01 was selected as the template for VL. The CDRs of murine antibody mAb1780 were grafted onto the selected humanized template; the FR amino acids were subjected to back-mutation. The light chain and heavy chain variable regions of the humanized antibodies were obtained, as shown in Table 7.

20

Table 7. Template selection and back-mutation design for h1780

h1780_VL		h1780_VH	
h1780_VL.1	Grafted	h1780_VH.1	Grafted
h1780_VL.1A	E1D, I2T	h1780_VH.1A	M70L, R72V, T74K
h1780_VL.1B	E1D, I2T, I57V, V84T, Y86F	h1780_VH.1B	M48I, V68A, M70L, R72V, T74K, L83F
		h1780_VH.1C	R38K, M48I, R67K, V68A, M70L, R72V, T74K, L83F

Note: “Grafted” represents that the murine antibody CDRs were grafted onto the human germline FR region. For example, according to the natural numbering of amino acid sequence, E1D represents that E at position 1 of “grafted” was mutated back to D.

- 5 The designed humanized molecules were combined to form different molecules shown in the following table, as shown in Table 8.

Table 8. Combination of h1780 humanized antibody heavy and light chain variable regions

	h1780 VH.1	h1780 VH.1A	h1780 VH.1B	h1780 VH.1C
h1780 VL.1	h1780-007	h1780-008	h1780-009	h1780-010
h1780 VL.1A	h1780-011	h1780-012	h1780-013	h1780-014
h1780 VL.1B	h1780-015	h1780-016	h1780-017	h1780-018

- 10 The specific sequences of the variable regions of the h1780 humanized antibody are as follows:

> h1780_VL.1 (SEQ ID NO: 60)

EIVLTQSPATLSLSPGERATLSCRASEGLTSYMHYQQKPGQAPRLLIYKASNLASGIPAR
FSGSGSGTDFTLTISLEPEDFAVYYCQQNWNDPWTFGGGTKVEIK

15

> h1780_VL.1A (SEQ ID NO: 61)

DTVLTQSPATLSLSPGERATLSCRASEGLTSYMHYQQKPGQAPRLLIYKASNLASGIPAR
FSGSGSGTDFTLTISLEPEDFAVYYCQQNWNDPWTFGGGTKVEIK

- 20 > h1780_VL.1B (SEQ ID NO: 62)

DTVLTQSPATLSLSPGERATLSCRASEGLTSYMHYQQKPGQAPRLLIYKASNLASGVPA
RFSGSGSGTDFTLTISLEPEDFATYFCQQNWNDPWTFGGGTKVEIK

> h1780_VH.1 (SEQ ID NO: 63)

- 25 EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYLIHWVRQAPGQGLEWMGYINPYSGGT

VYNEKFKSRVTMTRDTSISTAYMELSRLRSDDTAVYYCARDGGYSDPLDYWGQGTMTVT
VSS

> h1780_VH.1A (SEQ ID NO: 64)

5 EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYLIHWVRQAPGQGLEWMGYINPYSGGT
VYNEKFKSRVTLTVDKSISTAYMELSRLRSDDTAVYYCARDGGYSDPLDYWGQGTMTVT
VSS

> h1780_VH.1B (SEQ ID NO: 65)

10 EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYLIHWVRQAPGQGLEWIGYINPYSGGT
VYNEKFKSRATLTVDKSISTAYMEFSRLRSDDTAVYYCARDGGYSDPLDYWGQGTMTVT
VSS

> h1780_VH.1C (SEQ ID NO: 66)

15 EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYLIHWVKQAPGQGLEWIGYINPYSGGT
VYNEKFKSKATLTVDKSISTAYMEFSRLRSDDTAVYYCARDGGYSDPLDYWGQGTMTVT
VSS.

Each of the light chain variable regions as described above was combined with the light chain
20 constant region to form a light chain sequence. Each of the heavy chain variable regions was
combined with the heavy chain constant region to form a heavy chain sequence.

5.4 The selection of humanized framework for hybridoma clone mAb1773

For h1773, IGHV3-73*01 was selected as the template for VH, and IGKV1-39*01 was selected
25 as the template for VL. The CDRs of murine antibody mAb1773 were grafted onto the selected
humanized template; the amino acids were subjected to back-mutation. The light chain and
heavy chain variable regions of the humanized antibodies were obtained, as shown in Table 9. In

addition, N in h1773 HCDR2 (RIDPANGDTK HGPKFQG) was mutated into V (i.e. N55V) to form heavy chain variable region HCDR2 variant (the sequence of mutated HCDR2 is as shown in SEQ ID NO: 82: RIDPAVGDTKHGPKFQG).

5 Table 9. Template selection and back-mutation design for h1773

h1773_VL		h1773_VH	
h1773_VL.1	Grafted	h1773_VH.1	Grafted
h1773_VL.1A	M4L, A42S, L45P, L46W	h1773_VH.1A	F29I, R72A, T97F + N55V
		h1773_VH.1B	F29I, R38K, V48I, R72A, T97F + N55V

Note: “Grafted” represents that the murine antibody CDRs were grafted onto the human germline FR region. For example, according to the natural numbering of amino acid sequence, M4L represents that M at position 4 of “grafted” was mutated back to L.

10 The designed humanized molecules were combined to form different molecules shown in the following table, as shown in Table 10.

Table 10. Combination of h1773 humanized antibody heavy and light chain variable regions

	h1773_VH.1	h1773_VH.1A	h1773_VH.1B
h1773_VL.1	h1773-002	h1773-003	h1773-004
h1773_VL.1A	h1773-005	h1773-006	h1773-007

15 The specific sequences of the variable regions of the h1773 humanized antibody are as follows:

> h1773_VL.1 (SEQ ID NO: 67)

DIQMTQSPSSLSASVGDRVTITCSASSSVNYIYWYQQKPGKAPKLLIYLTATLASGVPSRF
SGSGSGTDFTLTISLQPEDFATYYCQQWNSYPYTFGGGTKVEIK

20

> h1773_VL.1A (SEQ ID NO: 68)

DIQLTQSPSSLSASVGDRVTITCSASSSVNYIYWYQQKPGKSPKPWIYLTATLASGVPSRFS
GSGSGTDFLTISLQPEDFATYYCQQWNSYPYTFGGGTKVEIK

5 > h1773_VH.1 (SEQ ID NO: 69)

EVQLVESGGGLVQPGGSLKLSCAASGFTFSNTYIHWVRQASGKGLEWVGRIDPAVGDTK
HGPKFQGRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRYGIYPDHWGQGTLVTVSS

> h1773_VH.1A (SEQ ID NO: 70)

10 EVQLVESGGGLVQPGGSLKLSCAASGFTISNTYIHWVRQASGKGLEWVGRIDPAVGDTK
HGPKFQGRFTISADDSKNTAYLQMNSLKTEDTAVYYCFRYGIYPDHWGQGTLVTVSS

> h1773_VH.1B (SEQ ID NO: 71)

15 EVQLVESGGGLVQPGGSLKLSCAASGFTISNTYIHWVKQASGKGLEWIGRIDPAVGDTK
HGPKFQGRFTISADDSKNTAYLQMNSLKTEDTAVYYCFRYGIYPDHWGQGTLVTVSS.

Each of the light chain variable regions as described above was combined with the light chain constant region sequence as shown in SEQ ID NO: 53 to form the final complete light chain sequence. Each of the heavy chain variable regions was combined with the heavy chain constant region as shown in SEQ ID NO: 52 to form the final complete heavy chain sequence.

20

5.5 The selection of humanized framework for hybridoma clone mAb1779

For h1779, IGHV1-2*02 was selected as the template for VH, and IGKV1-33*01 was selected as the template for VL. The CDRs of murine antibody h1779 were grafted onto the selected humanized template; the amino acids were subjected to back-mutation. The light chain and heavy chain variable regions of the humanized antibodies were obtained, as shown in Table 11.

25

Table 11. Template selection and back-mutation design for h1779

h1779_VL		h1779_VH	
h1779_VL.1	Grafted	h1779_VH.1	Grafted + D89E
h1779_VL.1A	A43S	h1779_VH.1A	R72A,T74K + D89E
h1779_VL.1B	A43S, I48V, F71Y	h1779_VH.1B	M48I, V68A, R72A, T74K + D89E
		h1779_VH.1C	M48I, V68A, R72A, T74K, M81L, L83F + D89E
		h1779_VH.1D	R38K, M48I, R67K, V68A, R72A, T74K, M81L, L83F + D89E

Note: “Grafted” represents that the murine antibody CDRs were grafted onto the human germline FR region. For example, according to the natural numbering of amino acid sequence, A43S represents that A at position 43 of “grafted” was mutated back to S.

5

The designed humanized molecules were combined to form different molecules shown in the following table, as shown in Table 12.

Table 12. Combination of h1779 humanized antibody heavy and light chain variable regions

	h1779_VH. 1	h1779_VH.1 A	h1779_VH.1 B	h1779_VH.1 C	h1779_VH.1 D
h1779_VL.1	h1779-005	h1779-006	h1779-007	h1779-008	h1779-009
h1779_VL.1 A	h1779-010	h1779-011	h1779-012	h1779-013	h1779-014
h1779_VL.1B	h1779-015	h1779-016	h1779-017	h1779-018	h1779-019

10

The specific sequences of the variable regions of the h1779 humanized antibody are as follows:

> h1779_VL.1 (SEQ ID NO: 72)

DIQMTQSPSSLSASVGDRVTITCLASEGISNDVAWYQQKPGKAPKLLIYAASRLQDGVPS

15 RFSGSGSGTDFTFTISLQPEDATYYCQQGYKTPLTFGQGTKLEIK

> h1779_VL.1A (SEQ ID NO: 73)

DIQMTQSPSSLSASVGDRVTITCLASEGISNDVAWYQQKPGKSPKLLIYAASRLQDGVPSR
FSGSGSGTDFTFTISSLQPEDIATYYCQQGYKTPLTFGQGGTKLEIK

5

> h1779_VL.1B (SEQ ID NO: 74)

DIQMTQSPSSLSASVGDRVTITCLASEGISNDVAWYQQKPGKSPKLLVYAASRLQDGVPS
RFSGSGSGTDYFTFTISSLQPEDIATYYCQQGYKTPLTFGQGGTKLEIK

10 > h1779_VH.1 (SEQ ID NO: 75)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIIHWVRQAPGQGLEWMGYFNPNSGGS
NYNENFKRRVTMTRDTSISTAYMELSRLRSEDVAVYYCARRIAWDHWYFDFWGQGTMTV
TVSS

15 > h1779_VH.1A (SEQ ID NO: 76)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIIHWVRQAPGQGLEWMGYFNPNSGGS
NYNENFKRRVTMTADKSISTAYMELSRLRSEDVAVYYCARRIAWDHWYFDFWGQGTMTV
TVSS

20 > h1779_VH.1B (SEQ ID NO: 77)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIIHWVRQAPGQGLEWIGYFNPNSGGS
NYNENFKRRATMTADKSISTAYMELSRLRSEDVAVYYCARRIAWDHWYFDFWGQGTMTV
TVSS

25 > h1779_VH.1C (SEQ ID NO: 78)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIIHWVRQAPGQGLEWIGYFNPNSGGS
NYNENFKRRATMTADKSISTAYLEFSRLRSEDVAVYYCARRIAWDHWYFDFWGQGTMTV

TVSS

>h1779_VH.1D (SEQ ID NO: 79)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIIHWVKQAPGQGLEWIGYFNPNSGGS
 5 NYNENFKRKATMTADKSISTAYLEFSRLRSEDYAVYYCARRIAWDHWYFDWVGQGMV
 TVSS.

Each of the light chain variable regions as described above was combined with the light chain constant region sequence as shown in SEQ ID NO: 53 to form a light chain sequence. Each of
 10 the heavy chain variable regions was combined with the heavy chain constant region as shown in
 SEQ ID NO: 52 to form a heavy chain sequence.

The full-length sequences of the exemplary humanized antibodies are as follows:

h1705-008 heavy chain:

15 EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVTSISYEGDITY
YGDSVKGRFTISRDN SKNTLYLQMNSLRAEDTATYYCASQTLRESFDYWVGQGLTVTVSS
 ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
 PSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 20 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
 DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
 RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 83

h1705-008 light chain:

25 DIQMTQSPSSVSASVGDRVTITCRASQDIANYLSWYQQKPGKSPKLLIYGTSNLEVGVP
RFSGSRSGTDFLTISLQPEDFATYYCLQDKEFPRTFGGGTKVEIKRTVAAPSVFIFPPSDE
 QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLS

KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 84

h1706-009 heavy chain:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSHYMAWVRQAPGKGLEWVSSINYE
NSA
 5 YYGDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCATETLRESLDYWGQGT
MVTV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
 SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
 GPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 10 RDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 85

h1706-009 light chain:

15 DIQMTQSPSSVSASVGDRVTITCRASQDIGNYLSWYQQKPGKSPKLMISASNLEVG
VPS
RFSGSGSGTDYTLTISSLPEDFATYFCLQHKQFPRTEGGG
TKVEIKRTVAAPSVFIFPPSDE
 QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLS
 KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 86

20 h1780-017 heavy chain:

EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYLIHWVRQAPGQGLEWIGYINPYSGGT
VYNEKFKSRATLTVDKSISTAYMEFSRLRSDDTAVYYCARDGGYSDPLDYWGQGT
MVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
 25 GGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
 SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD

KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 87

h1780-017 light chain:

DTVLTQSPATLSLSPGERATLSCRASEGLTSYMHYQOKPGQAPRLLIYKASNLASGVPA
 5 RFSGSGSGTDFLTITSSLEPEDFATYFCQQNWNDPWTFGGGKVEIKRTVAAPSVFIFPPSD
 EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLT
 SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 88

h1773-007 heavy chain:

10 EVQLVESGGGLVQPGGSLKLSCAASGFTISNTYIHWVKQASGKGLEWIGRIDPAVGD
TKHGPKFQGRFTISADDSKNTAYLQMNSLKTEDTAVYYCFRYGIYPDHWGQGLTVTVSSAS
 TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
 YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
 VFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
 15 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
 QGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 89

h1773-007 light chain:

20 DIQLTQSPSSLSASVGDRVTITCSASSSVNYIYWYQOKPGKSPKPWIYLTATLASGVPSRFS
GSGSGTDFLTITSSLPEDFATYYCQQWNSYPYTFGGGKVEIKRTVAAPSVFIFPPSDEQL
 KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKA
 DYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 90

25 h1779-014 heavy chain:

EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIIHWVKQAPGQGLEWIGYFNPNSGGS
NYNENFKRKATMTADKSISTAYLEFSRLRSEDTAVYYCARRIAWDHWYFDWGGQTMV

TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
5 SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO: 91

h1779-014 light chain:

DIQMTQSPSSLSASVGDRVTITCLASEGISNDVAWYQQKPGKSPKLLVYAASRLQDGVPS
10 RFSGSGSGTDYFTFTISSLQPEDATYYCQQGYKTPITFGQGTKLEIK
RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 92;

- 15 The antibody Hu39D10 against IL5 in WO2012083370A1 was used as a positive control in the present disclosure, and the heavy chain and light chain sequences thereof are shown in SEQ ID NO: 80 and SEQ ID NO: 81, respectively.

The sequence for Hu39D10 heavy chain

- 20 EVQLVESGGGLVQPGGSLRLSCAASGGLSTSSNVNWIRQAPGKGLEWVGLIWSNGD
TDYNSAIKSRFTISRDTSKSTVYLQMNSLRRAEDTAVYYCAREYYGYFDYWGQGTLVTVSSAS
TKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYTCNVDHKPSNTKVDKRVERSKYGPCCPPCPAPEFLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQFNSTYRV
25 VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQQEEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGN
VFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 80

The sequence for Hu39D10 light chain

DIQMTQSPSSLSASVGDRVTITCLASEGISSYLAWYQOKPGKAPKLLIYGANSLOTGVPS
RFSGSGSATDYTLTISSLOPEDFATYYCQOSYKFPNTFGQGTKVEVKRTVAAPSVFIFPPSD
 5 EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTL
 SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 81.

Example 6. Preparation of recombinant chimeric antibody and humanized antibody

10 1. Molecular cloning of recombinant chimeric antibody

After the positive antibody molecules were obtained by screening hybridomas, the gene sequences encoding variable region were obtained by sequencing. The forward and reverse primers were designed on the basis of the obtained sequences, and the sequenced gene was used as a template to construct each antibody VH/VK gene fragment by PCR, and then inserted into
 15 the expression vector pHr (having signal peptide and hIgG1/hkappa constant region gene (CH1-Fc/CL) fragment) by homologous recombination to construct a recombinant chimeric antibody full-length expression plasmid VH-CH1-Fc-pHr/VL-CL-pHr, so as to obtain five chimeric antibodies Ch1705, Ch1706, Ch1780, Ch1773 and Ch1779.

20 2. Molecular cloning of humanized antibodies

Humanized antibody sequence was subjected to codon optimization, then the coding gene sequence with human codon-preference was obtained; primers were designed to construct each antibody VH/VK gene fragment by PCR, which was then inserted into the expression vector pHr (having signal peptide and hIgG1/hkappa constant region gene (CH1-Fc/CL) fragment) by
 25 homologous recombination to construct a humanized antibody full-length expression plasmid VH-CH1-Fc-pHr/VL-CL-pHr.

3. Expression and purification of recombinant chimeric antibody and humanized antibody

The plasmid expressing the antibody light or heavy chain was separately transfected into HEK293E cells. 6 days later, the expression supernatant was collected, centrifuged at high speed to remove impurities, and purified with protein A column. The column was washed with PBS until the A280 reading was decreased to baseline. The target protein was eluted with acidic elution buffer, pH3.0-pH3.5 and neutralized with 1M Tris-HCl, pH 8.0-9.0. The eluted sample was appropriately concentrated, and was further purified by gel chromatography Superdex200 (GE) pre-equilibrated with PBS to remove aggregates, monomer peaks were collected, and aliquoted for use.

The following test methods were used to verify the performance and beneficial effects of the antibodies of the present disclosure.

Biological evaluation of activity *in vitro*

Test Example 1. The binding of murine IL-5 antibody to IL-5 of different species by Biacore assay

The affinity of the murine IL-5 antibody to be tested with human IL-5 was measured by Biacore T200 (GE) instrument.

The protein A biosensor chip was used to affinity capture of the molecules to be tested, and then the antigen (recombinant human and cyno IL-5 prepared in Example 1) flowed through the surface of the chip, and the reaction signal was detected in real time with the Biacore T200 instrument to obtain the binding and dissociation curves. After the dissociation of each experimental cycle was completed, the biosensor chip was washed and regenerated with glycine-hydrochloric acid regeneration solution (pH 1.5). BIAevaluation version 4.1 GE software was used to fit the data against (1:1) Langmuir model, and the affinity value was obtained and shown in Table 13.

Table 13. Results of affinity of murine IL-5 antibodies with IL-5 of different species by BIAcore assay

Antigen	KD (M)				
	mAb1705	mAb1706	mAb1780	mAb1773	mAb1779
human IL-5	7.27E-11	3.83E-11	8.99E-11	1.29E-10	4.58E-10
cyno IL-5	2.05E-10	2.77E-10	3.12E-10	4.76E-10	9.98 E-9

- 5 This example proves that the antibodies mAb1705, mAb1706, mAb1780, mAb1773 and mAb1779 of the present disclosure have high affinity to IL-5 of different species (human, monkey).

Test Example 2. The affinity of humanized IL-5 antibody to IL-5 of different species by
Biacore assay

The affinity of the humanized IL-5 antibody to be tested with human IL-5 was measured by Biacore T200 (GE) instrument.

15 The protein A biosensor chip was used to affinity capture of the molecules to be tested, and then the antigen (prepared in Example 1) flowed through the surface of the chip, and the reaction signal was detected in real time with the Biacore T200 instrument to obtain the binding and dissociation curves. After the dissociation of each experimental cycle was completed, the biosensor chip was washed and regenerated with glycine-hydrochloric acid regeneration solution (pH 1.5). BIAevaluation version 4.1 GE software was used to fit the data against (1:1) Langmuir
 20 model, and the affinity value was obtained and shown in Table 14.

Table 14. Results of affinity of humanized IL-5 antibodies to human IL-5 by BIAcore assay

Antibody	KD(M)	Antibody	KD(M)
h1705-003	3.35E-09	h1706-003	1.89E-11
h1705-006	4.11E-09	h1706-006	1.73E-11
h1705-009	4.55E-09	h1706-009	5.45E-11

h1705-004	2.14E-11	h1780-017	7.78E-11
h1705-007	2.21E-11	h1773-007	2.07E-10
h1705-010	2.05E-11	h1779-014	4.12E-10
h1705-005	2.16E-11		
h1705-008	3.42E-11		
h1705-011	2.30E-11		

The results show that all the humanized IL-5 antibodies have high affinity to human IL-5.

Test Example 3. ELISA-based assay of murine IL-5 antibody to block the binding of IL-5 to IL-5 α receptor

To identify the ability of IL-5 antibody to block IL-5 from binding to the extracellular region of recombinantly expressed IL-5 α receptor protein, IL-5 (5 μ g/ml in PBS) was coated on an ELISA plate and incubated at 37°C for 1 hour; the liquid was removed, 200 μ l/well of 5% skimmed milk blocking solution diluted with PBS was added, and incubated for 2.5 hours in a 37°C incubator for blocking. After blocking, the blocking solution was removed and the plate was washed with PBST buffer (pH 7.4 PBS comprising 0.05% Tween-20) for 5 times; 25 μ l of 10 μ g/ml IL-5R α (in 1% BSA) labeled by a biotin labeling kit (Tojin Chemical, LK03) was added, and then 25 μ l of gradient-diluted antibody was added (the antibody competed with IL-5R α for the binding with IL-5) and incubated at 37°C for 1 hour. After the incubation, the reaction solution in the microtiter plate was removed, the plate was washed for 5 times with PBST, added with 50 μ l/well of Streptavidin-Peroxidase Polymer (Sigma, S2438-250UG) diluted with sample dilution solution at 1:600 and incubated for 1 hour at 37°C. The plate was washed for 5 times with PBST, 50 μ l/well of TMB chromogenic substrate (KPL, 52-00-03) was added and incubated at room temperature for 3-10min; 50 μ l/well of 1M H₂SO₄ was added to stop the reaction; NOVOSTar microplate reader was used to read the absorbance value at 450nm; the IC₅₀ value of IL-5 antibody to block the binding of IL-5 to IL-5R α was calculated. The results are shown in Table

15. The antibodies of the present disclosure can effectively inhibit the binding of IL-5 to its receptor.

Table 15. ELISA results of murine IL-5 antibody to block the binding of IL-5 to IL-5 α receptor

	mAb1705	mAb1706
IC50 (μ g/ml)	0.42	0.40

5

Test Example 4. FACS-based assay of IL-5 antibody to block the binding of IL-5 to IL-5 receptor

In order to identify the screened IL-5 antibody which can block the IL-5 receptor on cell surface, CHOS recombinant cell line that highly expresses two receptors of IL-5R α / β was constructed.

10 This experiment demonstrated that the IL-5 antibodies can block the binding of IL-5 to the recombinant IL-5 α / β receptor on the surface of the CHOS cell line, respectively.

Particular method was as follows: CD-CHO comprising 100ng/ml G418 and 25ng/ml zeozin was used to culture CHO-S-IL-5R α and β . During cell culture, the concentration should not exceed
 15 3×10^6 cells/ml. IL-5R α / β -CHOS cells in good condition were centrifuged (at 1000 rpm, 5min), and washed once with 10% FBS in PBS, and the cells were counted, the cell concentration was adjusted to 4×10^6 cells/ml, and 25 μ l was taken out and added into a round-bottom 96-well plate. The antibody to be tested was diluted with PBS solution comprising 10% FBS. The initial concentration was 200 μ g/ml, and diluted at 1:10 for 8 gradient dilutions. 25 μ l of 100 ng/ml IL-5
 20 labeled by a biotin labeling kit (Tojin Chemical, LK03) was added, and fully mixed with 50 μ l of diluted antibody at each concentration, and added into 96-well plate that has been added with cells, and incubated at 4°C for 1 hour. After the incubation, the sample was centrifuged at 4°C (400g, 5min), and the supernatant was removed, the plated was washed with 200 μ l of pre-cooled PBS by centrifugation, repeated for twice; PE-Avidin secondary antibody diluted at 1:1333 was
 25 added and incubated in the dark at 4°C for 40min, centrifuged at 4°C (400g, 5min); the supernatant was removed, 200 μ l of pre-cooled PBS was added to pipette the cells; the plate was

washed by centrifugation at 4°C for three times; 100µl of PBS was added, the plate was read on machine; the IC50 value of IL-5 antibody to block the binding of IL-5 to IL-5Rα/β was calculated according to the fluorescence signal value. The results are shown in Table 16 and Figure 1.

5

Table 16. Test results of IL-5 antibody to block the binding of IL-5 to IL-5Rα/β

Antibody	IgG	hu39D10	h1705-008	h1706-009	h1780-017	h1779-014
IC50 (ng/ml)	8777	25.07	14.51	24.74	16	49.64

The results show that the antibodies h1705-008, h1706-009, h1780-017, and h1779-014 show a strong ability to block the binding of IL-5 to IL-5 receptor on cell surface.

10

Test Example 5. IL-5 antibody inhibits IL-5 induced proliferation of TF1 cells

IL-5 can induce the proliferation of TF-1 cells, and the IL-5 antibody can prevent IL-5 from stimulating the proliferation of TF-1 cells.

In particular: TF-1 cells (ATCC, CRL-2003) were cultured in RPMI1640 comprising 10% FBS and 2ng/mL rhGM-CSF (LinkBio, Catalog No. 96-AF-300-03-20), placed in 37 °C, 5% CO₂ incubator, the cell density would not exceed 1×10⁶ cells/ml. To detect the antibodies, cells at logarithmic growth phase were washed with PBS for three times and centrifuged at 800 rpm for 5 min; the cell density was adjusted to 6000 cells/well/90µl with RPMI1640 (FBS: 2%, recombinant human IL-5: 10ng/ml); 10µl of the gradient-diluted antibody to be tested was added to a 96-well plate for culture, after 3 days of culture, 30µl of cell titer was added and mixed for detection, IC50 was calculated according to the readings. The test results are shown in Table 17 below.

25

Table 17. Test results of IL-5 humanized antibody to inhibit the IL-5 induced TF1 cell proliferation

Antibody	IC50 (nM)	Antibody	IC50 (nM)
Hu39D10	0.30	h1706-003	0.31
h1705-004	0.30	h1706-004	0.30
h1705-005	0.30	h1706-006	0.34
h1705-007	0.25	h1706-007	0.28
h1705-008	0.20	h1706-009	0.25
h1705-010	0.30	h1773-007	0.38
h1705-011	0.28	h1780-017	0.16
		h1779-014	0.20

Test Example 6. Test of IL5 antibody to inhibit the IL5-induced eosinophil adhesion

- 5 IL5 can induce the differentiation, maturation, migration and activation of eosinophils, causing inflammation of the respiratory and leading to asthma. This experiment is based on the principle that IL-5 cytokines can promote the adhesion and activation of eosinophils. The eosinophils were collected and purified from human peripheral blood to test the blocking effect of IL-5 specific antibodies on IL-5 pathway, and to detect the blocking effect of IL-5 antibodies on IL5-mediated
- 10 eosinophil adhesion *in vitro*.

In particular: human peripheral blood was 5-fold diluted with PBS comprising 2mM EDTA, and Percoll™ (density gradient of 1.088) was used to isolate monocytes and granulocytes. The red blood cell layer comprising granulocytes was carefully aspirated, and red blood cell lysis

15 solution was used to remove red blood cells; the remaining cells were counted, the separation magnetic beads (Miltenyi Biotec, Catalog No. 130-045-701) with CD16 antibody were added in proportion, and incubated for 30 min and flowed through the magnetic bead column, the subpopulations (mainly eosinophils) directly flowing through the column were collected by negative selection. The isolated eosinophils were counted and added to a 96-well cell culture

20 plate pre-coated with IgG antibody, with about 1×10^4 cells per well; human IL-5 (20 ng/ml) and IL-5 antibody molecules at different concentrations (starting from 10 µg/ml, 3-fold dilution, 10 concentration points) were added; the cell culture plate was placed in 37°C, 5% CO₂ incubator

and incubated for 1 hour, then the culture plate was taken out and 0.3 % CTAB was added to lyse the cells, and finally the peroxidase reaction substrate TMB was added for color development, and the OD450 absorption value was read with a microplate reader. The reading value for the well added with IL-5 alone was the maximum absorption value; the well without IL-5 and antibody agent was served as background control; the inhibition value of antibody agent at each concentration relative to the maximum adsorption value was calculated = (maximum adsorption value-[antibody agent])/(maximum adsorption value-background control value) $\times$ 100%, and the IC50 was calculated. The results are shown in Table 18:

Table 18. IL-5 antibody blocks IL-5 induced eosinophil adhesion

	Hu39D10	h1705-008	h1706-009	h1780-017
IC50 (ng/ml)	11.79	4.85	4.3	21.19

The results show that the humanized antibodies of the present disclosure show a strong ability to inhibit IL5-mediated eosinophil adhesion.

Test Example 7. Evaluation of specificity of humanized IL-5 antibody with Th2 Cytokine

IL-5 was one of Th2 cytokines. In order to verify that IL-5 antibody only specifically targets IL-5 and does not cross-react with other cytokines, Fortebio was used to detect 12 types of Th2 and related cytokines, comprising IL2 (R&D, 202-IL-010/CF), IL4 (R&D, 204-IL-050/CF), IL-5 (R&D, 205-IL-025/CF), IFN γ , IL6 (R&D, 7270-IL-025/CF), IL9 (R&D, 209-IL-010/CF), IL10 (R&D, 217-IL-025/CF), IL13 (R&D, 213-ILB-025/CF), IL25 (R&D, 8134-IL-025/CF), IL31 (R&D, 2824-IL-010/CF), and IL3 (203-IL-050/CF) and GMCSF (R&D, 215-GM-010/CF) that share α receptors with IL-5.

In particular: Protein A Biosensor (PALL Fortebio, 18-5010) was used to capture the antibody, the capture signal was recorded, and then 40nM each cytokine was injected, the new binding signal was recorded. Finally, the binding signal with IL-5 was defined as 100%. The binding

signals of other cytokines with antibodies were observed, and the results are shown in Figure 2.

The results show that, among 12 related cytokines, humanized IL-5 antibodies h1705-008 and h1706-009 only specifically bind to IL-5, and have no cross-reactivity with other Th2 cytokines.

5

Biological evaluation of *in vivo* activity

Test Example 8. Evaluation of the efficacy of IL-5 antibody in OVA-induced mouse asthma model

10 This test was based on airway inflammatory response and airway remodeling to evaluate the efficacy of IL-5 antibody in BALB/c mouse asthma model induced by ovalbumin (OVA) aerosol.

The mice were randomly divided into 7 groups according to body weight, each group with 10 mice: normal control group (G1); model group (G2); the treatment groups of two antibodies to be tested h1705-008 (G3 and G4) and h1706-009 (G5 and G6) at two doses (10mpk and 2mpk) of each antibody to be tested; and positive antibody Hu39D10 control group (G7, 10mpk). On days 1 and 14, all mice were sensitized by intraperitoneal injection of allergenic solution. On days 28, 29, and 30, the six groups of mice (except the first group) were challenged by aerosol OVA challenge solution for 30 minutes. Two hours before the challenge, the second group (G2) was intraperitoneally injected with phosphate buffer, mice in the third group to the seventh group (G3-G7) were intraperitoneally injected with different doses of different antibodies (once a day, for three consecutive days). The antibodies to be tested were freshly prepared before each injection, and the administration was finished within half an hour since the preparation of antibody. Mice in the first group (as normal control group) were challenged with PBS aerosol for 30 minutes, and 2 hours before the challenge phosphate buffer was injected intraperitoneally once a day for three consecutive days.

On day 31, the WBP system was used to test the airway hyperresponsiveness of the animals. All animals were administered by aerosol to intake methacholine at 2-fold incremental concentrations (1.5625, 3.125, 6.25, 12.5, 25 and 50 mg/mL), the values of respiratory enhanced pause at corresponding concentrations were measured.

5

On day 31, 1 hour after the detection of airway responsiveness by WBP system, a tracheal tube with a diameter of 1.2 mm was inserted into trachea and fixed, and lung lavage was performed twice, each with 0.8 ml phosphate buffer comprising 1% BSA and 0.6mM EDTA. The recovery volume of lavage fluid was recorded.

10

The BALF was centrifuged at 300g at 4 degrees Celsius for 5 minutes. The supernatant was maintained for cytokine analysis. After centrifugation, the cells were resuspended in 1.5 ml of PBS (comprising 1% BSA and 0.6 mM EDTA) for cell counting. Hemocytometer and trypan blue staining experiment were used to count the total number of cells in BALF. The cells were smeared on silde, and stained with Wright staining solution for one minute, and then stained with Giemsa for 7 minutes to distinguish eosinophils, neutrophils, macrophages and lymphocytes. Counting was performed under a light microscope.

15

After lavage, the lung tissue was collected and stained with 10% neutral formaldehyde solution, and then fixed in 10% neutral formaldehyde solution. The fixed tissue was embedded in paraffin, trimmed, stained by H&E and scored. The test results are shown in Figure 3, Figure 4A and Figure 4B.

20

The results show that the antibody molecules h1705-008 and h1706-009 of the present disclosure can significantly improve lung function in a dose-dependent mode, while high dose (10mpk) of the positive compound cannot improve lung function (see Figure 3). Meanwhile, the two antibodies significantly reduce the level of eosinophils and the thickness of the mucous

25

membrane at the same dose (10mpk), and show a stronger ability to reduce eosinophils than that of the positive antibody (see Figures 4A and 4B). In the same type of mouse asthma model, repetitive experiments also verified that 1 mg/ml h1705-008, h1706-009 and h1780-017 significantly reduce the level of eosinophils in BalF than that of the positive antibody (see Figure 4C).

Test Example 9. Evaluation of the *in vivo* efficacy of IL-5 antibody in guinea pig acute asthma model induced by exogenous human IL-5

In this experiment, male guinea pigs were selected to establish acute asthma model induced by human IL-5, to evaluate the inhibitory effect of five IL-5 humanized mAbs of the present disclosure on the increase of eosinophils in bronchial lavage fluid (BALF) of guinea pig lung induced by human IL-5; and hu39D10 was used as a positive antibody. The guinea pigs were divided into 9 groups, each with 8-10 animals: normal control group, model group, hu39D10 (1 mg/kg) group, h1705-008 (1 mg/kg) group, h1706-009 (1 mg/kg) group, h1780-017 (1 mg/kg) group, h1773-007 (1 mg/kg) group and h1779-014 (1 mg/kg) group. The guinea pigs in model group and administration groups were tracheally injected with 100 μ l of human IL5 (comprising 5 μ g of IL5 antigen) on day1 for irritation, respectively; and the normal control group was tracheally injected with PBS. The administration group was intraperitoneally injected with 1 mg/kg IL5 monoclonal antibody as described above, 2 hours before irritation, with the administration volume of 5ml/kg; the model group was administered with the corresponding IgG antibody; and the normal control group was intraperitoneally administered with PBS solvent. The guinea pigs were anesthetized 24 hours after the tracheal injection, to extract the lung bronchial lavage fluid. The cell concentration was adjusted to 5×10^6 /ml, 15 μ l was dropped on the slide and dried for fixing; HE staining was performed, and the numbers of total cells and of eosinophils were counted under 400-fold microscope, and the percentage of eosinophils was calculated. The results are shown in Figure 5A and Figure 5B, indicating that 5 humanized antibodies of the present disclosure significantly reduce the level of eosinophils in BALF.

Selection and stability evaluation of the ingredients in the formulation

Exemplary preparation process for the pharmaceutical composition (formulation) of antibody:

Step 1: a certain amount of purified anti-IL-5 antibody solution was taken, and an antibody-free
5 buffer (such as 30mM, pH5.5 acetic acid-sodium acetate buffer) was used to replace
solvent-exchange (preferably by ultrafiltration); at least 6-fold of volume was exchanged by
ultrafiltration membrane, and the protein was concentrated to about 120 mg/mL. A certain
volume of sucrose stock solution was added and mixed to get a final concentration of 72 mg/mL
of sucrose. A certain volume of polysorbate 80 stock solution was added and mixed to get a final
10 concentration of 0.4 mg/mL of polysorbate 80. 10mM pH 5.5 acetic acid-sodium acetate buffer
was added to reach a certain volume, resulting in the protein concentration of 100 mg/mL (other
formulations to be tested or stable formulations were prepared according to similar steps).

The product was filtered, and then was tested by central-control sampling for pathogenic
15 agents-free. The stock solution was passed through a 0.22 μ m PVDF filter, and the filtrate was
collected.

Step 2: the volume was adjusted to 1.2ml, the filtrate was loaded in 2ml vial applied with stopper,
and central-control samplings were taken at the beginning, in the middle, and at the end of
20 loading to detect the difference of loading volume.

Step 3: the capping machine was started to apply aluminum caps, and to perform capping.

Step 4: visual inspection was performed to confirm that products have no defects, such as
25 inaccurate loading. The vial labels were printed and attached; the carton labels were printed; the
cartons were folded; packing; and box labels were attached.

Test Example 10. Screening of buffer system

The h1705-008 formulations with a protein concentration of 100 mg/mL were prepared in a series of buffers at pH of 5.0 to 6.5, wherein the shaking sample comprised 0.2 mg/ml polysorbate 80 (PS80), and other samples comprised 0.05 mg/mL PS80. The buffer systems were as follows: 10mM acetic acid-sodium acetate (AA) pH5.0, 5.5; 10mM succinic acid-sodium succinate (SA) pH5.0, 5.5, 6.0; 10mM citric acid-sodium citrate (CA) pH5.5, 6.0, 6.5; 10mM histidine-hydrochloride (His) pH5.5, 6.0, 6.5; 10mM phosphate (PB) pH6.0, 6.5. Each formulation was filtrated, loaded, applied with stopper and capped. The samples were subjected to a forced degradation experiment; and appearance, SEC, iCIEF were served as evaluation indicators.

The results are shown in Table 19. Appearance data indicates that the samples experienced shaking (300rpm, 25°C) and samples at 40°C show different degrees of particle formation. Overall, the appearance is better when the pH is lower, and the buffer systems acetic acid-sodium acetate and succinic acid-sodium succinate are better; SEC data shows that AA pH 5.5 group is slightly better at 40°C; iCIEF data shows that AA pH 5.5, His pH 5.5, CA pH 6.5 groups are slightly better at 40°C; under comprehensive consideration of physical and chemistry stability, AA pH 5.5 is preferable.

Table 19. Screening results for pH and buffer system

Batch No.	Condition	Appearance	SEC (%)	iCIEF neutral peak (%)
01 AA5.0	T0	clear	98.3	75.3
	Shaking D4	clear	96.2	N/A
	40°C-D13	fine particles	94.8	62.3
02 AA5.5	T0	clear	98.0	75.4
	Shaking D4	clear	96.0	N/A
	40°C-D13	flocculent small particles	95.4	64.2
03 SA5.0	T0	clear	98.2	74.9
	Shaking D4	clear	96.5	N/A
	40°C-D13	fine particles+	94.8	59.3
04	T0	clear	98.2	74.7

SA5.5	Shaking D4	large amount of small particles	96.2	N/A
	40°C-D13	small particles	94.8	62.1
05 SA6.0	T0	clear	97.9	75.8
	Shaking D4	clear, slightly opalescence	95.7	N/A
	40°C-D13	small particles	95.0	63.7
06 His5.5	T0	clear	98.2	75.7
	Shaking D4	clear, few particles	96.2	N/A
	40°C-D13	small particles	94.9	64.8
07 His6.0	T0	clear	98.3	74.6
	Shaking D4	large amount of particles	96.2	N/A
	40°C-D13	medium particles	94.6	65.8
08 His6.5	T0	clear	98.1	74.6
	Shaking D4	severe opalescence	95.8	N/A
	40°C-D13	medium particles	94.3	62.3
09 CA5.5	T0	clear	98.2	74.8
	Shaking D4	clear, slightly opalescence	N/A	N/A
	40°C-D13	fine particles	94.7	60.9
10 CA6.0	T0	clear	98.0	75.3
	Shaking D4	obvious opalescence, large amount of particles	N/A	N/A
	40°C-D13	medium particles	94.6	61.5
11 CA6.5	T0	clear	97.9	74.6
	Shaking D4	severe opalescence, large amount of particles	N/A	N/A
	40°C-D13	small particles	95.0	64.4
12 PB6.0	T0	clear	98.0	74.4
	Shaking D4	with particles	N/A	N/A
	40°C-D13	fine particles	94.3	63.0
13 PB6.5	T0	clear	97.9	74.7
	Shaking D4	severe opalescence, large amount of particles	N/A	N/A
	40°C-D13	large particles	93.9	61.9

Note: N/A represents not detected, D represents the day, and T0 represents day 0.

Test Example 11. Screening of excipients in formulations

The h1705-008 formulations with a protein concentration of 100 mg/mL were prepared in 10 mM SA (pH 5.0) buffer comprising different types of excipients below. In particular, the excipients were as follows:

- 1) 0.1 mg/mL polysorbate 20 (PS20)

- 2) 0.1 mg/mL polysorbate 80 (PS80)
- 3) 50 mg/mL sucrose + 0.1 mg/mL PS80
- 4) 50 mg/mL trehalose + 0.1 mg/mL PS80
- 5) 50 mg/mL mannitol + 0.1 mg/mL PS80
- 5 6) 50 mg/mL sorbitol + 0.1 mg/mL PS80
- 7) 8 mg/ml arginine (Arg) + 0.1 mg/mL PS80
- 8) 8 mg/ml lysine (Lys) + 0.1 mg/mL PS80
- 9) 8 mg/ml glycine (Gly) + 0.1 mg/mL PS80
- 10) 8 mg/ml methionine (Met) + 0.1 mg/mL PS80
- 10 11) 8 mg/ml proline (Pro) + 0.1 mg/mL PS80
- 12) 8 mg/ml sodium chloride (NaCl) + 0.1 mg/mL PS80.

Each formulation was filtrated, loaded, applied with stopper and capped, for use. The samples were subjected to a forced degradation experiment at 40°C, the results show that (Table 20): there is no significant difference in the test results of SEC, CE, and iCIEF among each group of samples, Arg/Lys/NaCl groups have poorer appearance, and there is no significant difference among other groups. Sucrose, trehalose, mannitol, sorbitol, glycine, proline, and methionine have favorable effect on protein stability.

Table 20. The screening results of excipients

Batch No.	Condition	Appearance	SEC (%)	iCIEF neutral peak (%)	CE-SDS (%)
1	D0	clear	96.6	75.16	95.05
	40°C D12	clear	94.29	59.24	93.53
2	D0	clear	96.64	74.39	94.96
	40°C D12	clear	94.33	58.99	93.31
3	D0	clear	96.59	74.51	95.44
	40°C D12	clear	94.33	58.33	93.3
4	D0	clear	96.63	74.87	95.26
	40°C D12	clear	94.43	58.14	93.68
5	D0	clear	96.63	74.8	95.33
	40°C D12	clear	94.47	59.79	93.43
6	D0	clear	96.63	74.25	95.29
	40°C D12	clear	94.39	58.04	93.41

7	D0	clear, light opalescence	96.6	74.31	95.19
	40°C D12	opalescence, large amount of particles	94.27	59.18	93.45
8	D0	clear, light opalescence	96.56	74.7	95.2
	40°C D12	opalescence	94.26	59.62	93.56
9	D0	clear	96.54	73.77	95.47
	40°C D12	clear	94.38	58.82	93.56
10	D0	clear	96.56	75.68	95.5
	40°C D12	clear	94.45	59.57	93.71
11	D0	clear	96.43	75.22	95.02
	40°C D12	clear	94.47	61.1	95.32
12	D0	clear, opalescence	96.51	73.63	95.02
	40°C D12	opalescence, large amount of particles	93.99	60.51	93.21

Note: D represents day.

Test Example 12. Screening of Surfactants

The h1705-008 formulations comprising 10mM SA pH5.5, 70 mg/ml sucrose, 0.4 mg/ml PS20 or PS80 were prepared, with a protein concentration of 100 mg/ml.

The samples were placed at 4°C to investigate the stability. The results are shown in Table 21. The results show that PS80 group exhibits a clear appearance and no significant changes in SEC, CE, and iCIEF at 4°C for 4 months, indicating a favorable stability; whereas a large amount of particles are observed in PS20 group. Therefore, PS80 is better than PS20. In addition, it can be seen that the addition of PS80 into the formulation has a better stabilizing effect on h1705-008, and the stability of the formulation is better.

Table 21. stability results of h1705-008 at 4°C

Group	Time (M)	Appearance	SEC (%)	Non-reducing CE-SDS (%)	iCIEF neutral peak (%)
PS80	0	clear	97.2	94.4	71.2
	4	clear	97.6	95.0	71.6
PS20	0	clear	97.1	94.3	72.7
	4	large amount of particles	N/A	N/A	N/A

Note: M represents month; N/A represents not detected.

Test Example 13. The design and screening of DOE formulation

DOE (Design of experiment) was performed with pH of 10mM acetate buffer (AA), protein
 5 concentration and Tween concentration as variables; a series of formulations were designed
 based on the following factors and levels: pH is 5.0 to 5.8, the concentration of PS80 is 0.2 to 0.6
 mg/mL, concentration of the antibody h1705-008 is 80 to 120 mg/mL; The formulations are
 shown in Table 22. The samples were subjected to forced degradation at high temperature of
 40°C. Appearance, SEC, non-reducing CE, and iCIEF were used as evaluation indicators. The
 10 results are shown in Table 23.

Table 22. Screening experiment and design for DOE formulations

Batch No.	pH	PS80 (mg/mL)	The amount of protein (mg/mL)
1	5.8	0.2	80
2	5.4	0.6	100
3	5.4	0.4	80
4	5.0	0.2	80
5	5.4	0.2	100
6	5.8	0.6	80
7	5.0	0.2	120
8	5.0	0.6	80
9	5.8	0.6	120
10	5.4	0.4	120
11	5.4	0.4	100
12	5.0	0.6	120
13	5.8	0.2	120
14	5.0	0.4	100
15	5.8	0.4	100
16	5.4	0.4	100

Table 23. Screening experiment results of DOE formulations

Batch No.	Condition	Appearance	SEC (%)	iCIEF neutral peak (%)	Non-reducing CE-SDS (%)
1	D0	clear	98.9	63.9	93.8
	40°C D15	clear	97.8	49.9	92.5
2	D0	clear	98.9	64.5	93.8
	40°C D15	clear	97.5	49.8	92.9
3	D0	clear	98.9	64.3	94.0
	40°C D15	clear	97.6	49.6	92.2
4	D0	clear	99.0	63.6	93.9
	40°C D15	clear	97.7	50.3	92.4
5	D0	clear	98.9	63.4	93.8
	40°C D15	clear	97.6	49.7	92.2
6	D0	clear	98.9	63.5	93.9
	40°C D15	clear	97.7	51.5	92.4
7	D0	clear	98.9	64.3	93.7
	40°C D15	clear	97.6	49.2	92.4
8	D0	clear	99.0	64.4	93.9
	40°C D15	clear	97.7	49.6	92.3
9	D0	clear	98.7	66.2	93.8
	40°C D15	clear	97.3	50.6	92.4
10	D0	clear	98.8	64.1	93.9
	40°C D15	clear	97.4	50.9	92.3
11	D0	clear	98.8	64.5	94.0
	40°C D15	clear	97.5	50.6	92.4
12	D0	clear	99.0	64.4	93.7
	40°C D15	clear	97.6	49.9	92.3
13	D0	clear	98.8	63.7	93.8
	40°C D15	clear	97.4	50.8	92.6
14	D0	clear	99.0	63.6	93.9
	40°C D15	clear	97.7	50.9	92.1
15	D0	clear	98.8	63.0	93.6
	40°C D15	clear	97.6	51.1	92.4
16	D0	clear	98.8	64.9	93.8
	40°C D15	clear	97.5	50.0	92.3

Note: D represents day.

The results show that the appearance of each formulation is clear; SEC, CE and iCIEF are decreased within an acceptable range, by <2%, <2% and about 14%, respectively; and the stability of formulation is favorable; therefore the formulation comprises a protein concentration of 80-120 mg/ml, 0.2-0.6 mg/ml PS80, pH 5.0-5.8. The optimal formulation is: 100 mg/ml protein, 0.4 mg/ml PS80, pH 5.5.

Test Example 14. Stability test

The h1705-008 formulations comprising 10 mM AA pH 5.5, 70 mg/ml sucrose, and 0.4 mg/ml PS80 were prepared, with a protein concentration of 100 mg/ml; and the samples were subjected to stability investigation at 4 °C and 25 °C. The results are shown in Table 24.

The results show that under high temperature conditions, SEC, CE, and iCIEF are slightly decreased in h1705-008 formulation, but the decrease is within an acceptable range; there is no significant change in all indicators under other conditions. The formulation has favorable stability, and can ensure the stability of h1705-008 at 4 °C within 6 months.

Table 24. Stability results of h1705-008 at 25°C and 4°C

Condition	Appearance	SEC (%)	iCIEF neutral peak (%)	Non-reducing CE-SDS (%)
T0	clear	98.0	59.8	94.5
25°C - M3	clear	97.6	55.2	93.4
25°C - M6	clear	96.7	51.7	91.7
4°C - M3	clear	98.5	59.7	94.6
4°C - M6	clear	98.7	60.0	94.0

Note: M represents month.

Test Example 15. Screening of ionic strength

The h1705-008 formulations comprising protein concentration of 100 mg/mL, 70 mg/mL sucrose and 0.4 mg/mL PS80 were prepared in (sodium) acetate buffer with different ionic strengths; the

pH of buffers used for exchange and the pH of the final formulations were measured. The results are shown in Table 25. The results show that the higher the ionic strength, the lower the pH drift. When the ionic strength is 30mM, the pH drift is less than 0.1.

5 Table 25. pH results of formulations with different ionic strengths

Ionic strength	Buffer - pH	Stock solution of the formation - pH	Δ pH
10mM	5.50	5.71	0.21
20mM	5.50	5.66	0.16
30mM	5.50	5.59	0.09

Test Example 16. Screening of concentration of saccharide

The h1705-008 formulations comprising a protein concentration of 100 mg/mL, 30 mM AA pH 5.5, 0.4 mg/ml PS80 were prepared in the following buffers comprising sucrose with different concentrations. The osmotic pressure was determined. The results are shown in Table 26.

The results show that the osmotic pressure is in an optimal isotonic range of 290 to 310 mosm, when the saccharide concentration is 70-75 mg/ml; according to the osmotic pressure data of 70 mg/ml and 73 mg/ml groups, the osmotic pressure reaches the best value of about 300 mosm, when the saccharide concentration is 72 mg/ml.

Table 26. Comparison of osmotic pressure in h1705-008 formulations with different saccharide concentrations

Concentration of saccharide (mg/ml)	70	73	75
Osmotic pressure (mosm)	290	306	310

20 Test Example 17. Stability test of the formulations

The h1705-008 formulations comprising a protein concentration of 100 mg/mL, 30mM AA pH 5.5, 72 mg/ml sucrose, 0.4 mg/ml PS80 were prepared to investigate the stability at 4°C and

25°C. The results are shown in Table 27. The results show that, SEC, CE and IEC are slightly decreased in the h1705-008 formulation under high temperature conditions, but the decrease is within an acceptable range; there is no significant change in all indicators at 4°C condition, indicating that the formulations have favorable stability.

5

Table 27. Stability of h1705-008 formulations

Condition	Appearance	SEC (%)	IEC neutral peak (%)	Non-reducing CE-SDS (%)
T0	clear	98.4	63.5	97.6
25°C - M3	N/A	96.8	55.0	96.5
4°C - M3	clear	98.4	61.9	97.3

Note: M represents month, T represents time, N/A represents not determined.

Test Example 18. Additional optional formulations

10 In addition, the present disclosure also provides additional formulations for the anti-IL-5 antibody pharmaceutical formulations, comprising but not limited to:

(1) 1 mg/ml anti-IL-5 antibody (h1705-008), 72 mg/ml sucrose, 0.4 mg/ml polysorbate 80, and 10 mM acetic acid-sodium acetate buffer at pH 5.5;

(2) 100 mg/ml anti-IL-5 antibody (h1705-008), 72 mg/ml sucrose, 0.4 mg/ml polysorbate 80, and
15 20 mM acetic acid-sodium acetate buffer at pH 5.5;

(3) 120 mg/ml anti-IL-5 antibody (h1705-008), 72 mg/ml sucrose, 0.4 mg/ml polysorbate 80, and 40 mM acetic acid-sodium acetate buffer at pH 5.5;

(4) 100 mg/ml anti-IL-5 antibody (h1705-008), 80 mg/ml sucrose, 0.6 mg/ml polysorbate 80, and 30 mM acetic acid-sodium acetate buffer at pH 5.0;

20 (5) 80 mg/ml anti-IL-5 antibody (h1705-008), 75 mg/ml sucrose, 0.6 mg/ml polysorbate 80, and 20 mM acetic acid-sodium acetate buffer at pH 5.4;

(6) 100 mg/ml anti-IL-5 antibody (h1705-008), 80 mg/ml sucrose, 0.4 mg/ml polysorbate 80, and 30 mM acetic acid-sodium acetate buffer at pH 5.5;

(7) 90 mg/ml anti-IL-5 antibody (h1705-008), 74 mg/ml sucrose, 0.5 mg/ml polysorbate 80, and

- 25 mM acetic acid-sodium acetate buffer at pH 5.6;
- (8) 90 mg/ml anti-IL-5 antibody (h1705-008), 76 mg/ml sucrose, 0.3 mg/ml polysorbate 80, and 35 mM acetic acid-sodium acetate buffer at pH 5.4;
- (9) 80 mg/ml anti-IL-5 antibody (h1705-008), 72 mg/ml sucrose, 0.4 mg/ml polysorbate 80, and 30 mM acetic acid-sodium acetate buffer at pH 5.6;
- (10) 100 mg/ml anti-IL-5 antibody (h1705-008), 72 mg/ml sucrose, 0.4 mg/ml polysorbate 80, and 40 mM acetic acid-sodium acetate buffer at pH 5.5;
- (11) 100 mg/ml anti-IL-5 antibody (h1705-008), 80 mg/ml sucrose, 0.4 mg/ml polysorbate 80, and 40 mM acetic acid-sodium acetate buffer at pH 5.5.
- 10 The experimental results show that the IL-5 antibody formulations as described above have favorable stability and can be applied to the preparation of IL-5 antibody agents.

Test Example 19. Lyophilization of anti-IL-5 antibody formulations

The h1705-008 antibody formulations comprising 72 mg/ml sucrose, 0.4 mg/ml polysorbate 80 and a concentration of 100 mg/ml anti-IL-5 antibody were prepared in 30mM acetic acid-sodium acetate buffer at pH 5.5. The antibody was loaded into 6 mL vial at 2.15 mL/vial, and was placed in a lyophilization chamber for lyophilization. The lyophilization process comprises pre-freezing, primary drying and secondary drying. When the lyophilization process was over, the vials were subjected to vacuum and applied with stoppers. The reconstituted samples were compared to the counterpart before the lyophilization. The results show that the reconstituted solutions can maintain favorable performance as that of the liquid formulations.

Table 28. Lyophilization steps of the formulations

Process parameters for lyophilization	The set of temperature (°C)	The degree of vacuum (mBar)
pre-freezing	5	N/A
	-45	N/A
primary drying	-27	0.1
secondary drying	25	0.1
	25	0.01

Note: N/A represents that the table was not applicable.

What claimed is:

1. A pharmaceutical composition, comprising an anti-IL-5 antibody or an antigen-binding fragment thereof, a buffer and a surfactant, wherein the buffer is any one selected from the group consisting of acetic acid-sodium acetate, succinic acid-sodium succinate, histidine-hydrochloride and citric acid-sodium citrate buffer, preferably acetic acid-sodium acetate or succinic acid-sodium succinate buffer; wherein, the anti-IL-5 antibody or the antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region selected from the group consisting of i) to vi):
 - i) a heavy chain variable region comprising HCDR1, HCDR2 and HCDR3 as shown in amino acid sequence SEQ ID NOS: 16, 17 and 18, respectively, and a light chain variable region comprising LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOS: 19, 20 and 21, respectively;
 - ii) a heavy chain variable region comprising HCDR1, HCDR2 and HCDR3 as shown in amino acid sequence SEQ ID NOS: 22, 23 and 24, respectively, and a light chain variable region comprising LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOS: 25, 26 and 27, respectively;
 - iii) a heavy chain variable region comprising HCDR1, HCDR2 and HCDR3 as shown in amino acid sequence SEQ ID NOS: 28, 29 and 30, respectively, and a light chain variable region comprising LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOS: 31, 32 and 33, respectively;
 - iv) a heavy chain variable region comprising HCDR1, HCDR2 and HCDR3 as shown in amino acid sequence SEQ ID NOS: 34, 35 and 36, respectively, and a light chain variable region comprising LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOS: 37, 38 and 39, respectively;
 - v) a heavy chain variable region comprising HCDR1, HCDR2 and HCDR3 as shown in amino acid sequence SEQ ID NOS: 40, 41 and 42, respectively, and a light chain variable region comprising LCDR1, LCDR2 and LCDR3 as shown in amino acid sequence SEQ ID NOS: 43, 44 and 45, respectively; and
 - vi) a heavy chain variable region comprising HCDR1, HCDR2 and HCDR3 as shown in amino acid sequence SEQ ID NOS: 34, 82 and 36, respectively, and a light chain variable region comprising LCDR1, LCDR2 and LCDR3 as shown in amino acid

sequence SEQ ID NOs: 37, 38 and 39, respectively.

2. The pharmaceutical composition according to claim 1, wherein the pH of the buffer is about 5.0 to about 6.5, preferably about 5.0 to about 5.8, and most preferably about 5.5.

5

3. The pharmaceutical composition according to claim 1 or 2, wherein the concentration of the buffer is about 10 mM to about 40 mM, preferably about 20 mM to about 30 mM.

4. The pharmaceutical composition according to any one of claims 1 to 3, wherein the
10 concentration of the anti-IL-5 antibody or the antigen-binding fragment thereof is about 1 mg/ml to about 120 mg/ml, preferably about 80 mg/ml to about 120 mg/ml, and most preferably about 100 mg/ml.

5. The pharmaceutical composition according to any one of claims 1 to 4, wherein the surfactant
15 is polysorbate 80.

6. The pharmaceutical composition according to claim 5, wherein the concentration of the polysorbate 80 is about 0.1 mg/ml to about 0.6 mg/ml, preferably about 0.2 mg/ml to about 0.6 mg/ml, and more preferably about 0.4 mg/ml.

20

7. The pharmaceutical composition according to any one of claims 1 to 6, further comprising a stabilizer, wherein the stabilizer is a saccharide or an amino acid, wherein the saccharide is selected from the group consisting of sucrose, trehalose, mannitol and sorbitol, preferably sucrose; wherein the amino acid is selected from the group consisting of glycine, methionine and
25 proline.

8. The pharmaceutical composition according to claim 7, wherein the concentration of the saccharide is about 50 mg/ml to about 80 mg/ml, preferably about 70 mg/ml to about 75 mg/ml, and most preferably about 72 mg/ml.

30

9. The pharmaceutical composition according to claim 7, wherein the concentration of the amino acid is about 8 mg/ml.

10. The pharmaceutical composition according to any one of claims 1 to 9, which comprises the following components:

about 1 mg/ml to about 120 mg/ml the anti-IL-5 antibody or the antigen-binding fragment thereof;

about 10mM to about 40mM acetic acid-sodium acetate buffer, pH is about 5.0 to about 6.5; and about 0.1 mg/ml to about 0.6 mg/ml polysorbate 80.

11. The pharmaceutical composition according to any one of claims 1 to 9, which comprises:

about 80 mg/ml to about 120 mg/ml the anti-IL-5 antibody or the antigen-binding fragment thereof,

about 10mM to about 30mM acetic acid-sodium acetate buffer, pH is about 5.0 to about 5.8,

about 0.2 mg/ml to about 0.6 mg/ml polysorbate 80, and

about 70 mg/ml to about 80 mg/ml sucrose;

preferably, the pharmaceutical composition comprises:

about 100 mg/ml the anti-IL-5 antibody or the antigen-binding fragment thereof,

about 30 mM acetic acid-sodium acetate buffer, pH is about 5.5,

about 0.4 mg/ml polysorbate 80, and

about 72 mg/ml sucrose.

12. The pharmaceutical composition according to any one of claims 1 to 11, wherein the anti-IL-5 antibody or the antigen-binding fragment thereof is a murine antibody, a chimeric antibody or a humanized antibody.

13. The pharmaceutical composition according to claim 12, wherein the humanized anti-IL-5 antibody comprises a heavy chain variable region as shown in SEQ ID NO: 49, 57, 63, 69 or 75 or variant thereof; the variant comprises 1 to 10 amino acid back-mutations in the heavy chain variable region sequence as shown in SEQ ID NO: 49, 57, 63, 69 or 75, respectively.

14. The pharmaceutical composition according to claim 13, wherein the variant is a variant as shown in any one of the followings:

- i) a variant, comprising one or more amino acid back-mutations selected from the group consisting of S49T, V93T and K98S in the heavy chain variable region as shown in SEQ ID NO: 49;
- ii) a variant, comprising one or more amino acid back-mutations selected from the group consisting of S49T, V93T and K98T in the heavy chain variable region as shown in SEQ ID NO: 57;
- iii) a variant, comprising one or more amino acid back-mutations selected from the group consisting of R38K, M48I, R67K, V68A, M70L, R72V, T74K and L83F in the heavy chain variable region as shown in SEQ ID NO: 63;
- iv) a variant, comprising one or more amino acid back-mutations selected from the group consisting of F29I, R38K, V48I, R72A, and T97F in the heavy chain variable region as shown in SEQ ID NO: 69, and/or N55V mutation in HCDR2; or
- v) a variant, comprising one or more amino acid back-mutations selected from the group consisting of R38K, M48I, R67K, V68A, R72A, T74K, M81L, L83F and D89E in the heavy chain variable region as shown in SEQ ID NO: 75.

15. The pharmaceutical composition according to claim 14, wherein the humanized anti-IL-5 antibody comprises:

- a heavy chain variable region as shown in SEQ ID NO: 50 or 51; or
- a heavy chain variable region as shown in SEQ ID NO: 58 or 59; or
- a heavy chain variable region as shown in any one selected from the group consisting of: SEQ ID NOs: 64, 65 and 66; or
- a heavy chain variable region as shown in SEQ ID NO: 70 or 71; or
- a heavy chain variable region as shown in any one selected from the group consisting of: SEQ ID NOs: 76, 77, 78 and 79.

16. The pharmaceutical composition according to claim 12, wherein the humanized anti-IL-5 antibody comprises a light chain variable region as shown in SEQ ID NO: 46, 54, 60, 67 or 72, or variant thereof; the variant comprises 1 to 10 amino acid back-mutations in the light chain

variable region as shown in SEQ ID NO: 46, 54, 60, 67 or 72.

17. The pharmaceutical composition according to claim 16, wherein the variant is a variant as shown in any one of the followings:

- 5 i) a variant, comprising one or more amino acid back-mutations selected from the group consisting of A43S, L47V, G66R, T69S, F71Y and Y87F in the light chain variable region as shown in SEQ ID NO: 46;
- ii) a variant, comprising one or more amino acid back-mutations selected from the group consisting of A43S, L47M, F71Y and Y87F in the light chain variable region as shown in SEQ
10 ID NO: 54;
- iii) a variant, comprising one or more amino acid back-mutations selected from the group consisting of E1D, I2T, I57V, V84T and Y86F in the light chain variable region as shown in SEQ ID NO: 60;
- iv) a variant, comprising one or more amino acid back-mutations selected from the group
15 consisting of M4L, A42S, L45P and L46W in the light chain variable region as shown in SEQ ID NO: 67; or
- v) a variant, comprising one or more amino acid back-mutations selected from the group consisting of A43S, I48V and F71Y in the light chain variable region as shown in SEQ ID NO:
72.

20

18. The pharmaceutical composition according to claim 17, wherein the humanized anti-IL-5 antibody comprises:

- a light chain variable region as shown in SEQ ID NO: 47 or 48; or
- a light chain variable region as shown in SEQ ID NO: 55 or 56; or
- 25 a light chain variable region as shown in SEQ ID NO: 61 or 62; or
- a light chain variable region as shown in SEQ ID NO: 68; or
- a light chain variable region as shown in SEQ ID NO: 73 or 74.

19. The pharmaceutical composition according to claim 12, wherein the humanized anti-IL-5
30 antibody comprises:

- i) a heavy chain variable region as shown in any one of SEQ ID NOs: 49, 50 and 51 or having 95%

- sequence identity with any one of SEQ ID NOs: 49, 50 and 51; and
a light chain variable region as shown in any one of SEQ ID NOs: 46, 47 and 48 or having 95% sequence identity with any one of SEQ ID NOs: 46, 47 and 48;
- 5 ii) a heavy chain variable region as shown in any one of SEQ ID NOs: 57, 58 and 59 or having 95% sequence identity with any one of SEQ ID NOs: 57, 58 and 59; and
a light chain variable region as shown in any one of SEQ ID NOs: 54, 55 and 56 or having 95% sequence identity with any one of SEQ ID NOs: 54, 55 and 56;
- 10 iii) a heavy chain variable region as shown in any one of SEQ ID NOs: 63, 64, 65 and 66 or having 95% sequence identity with any one of SEQ ID NOs: 63, 64, 65 and 66; and
a light chain variable region as shown in any one of SEQ ID NOs: 60, 61 and 62 or having 95% sequence identity with any one of SEQ ID NOs: 60, 61 and 62;
- iv) a heavy chain variable region as shown in any one of SEQ ID NOs: 69, 70 and 71 or having 95% sequence identity with any one of SEQ ID NOs: 69, 70 and 71; and
a light chain variable region as shown in any one of SEQ ID NOs: 67 and 68 or having 95% sequence identity with any one of SEQ ID NOs: 67 and 68; or
- 15 v) a heavy chain variable region as shown in any one of SEQ ID NOs: 75, 76, 77, 78 and 79 or having 95% sequence identity with any one of SEQ ID NOs: 75, 76, 77, 78 and 79; and
a light chain variable region as shown in any one of SEQ ID NOs: 72, 73 and 74 or having 95% sequence identity with any one of SEQ ID NOs: 72, 73 and 74;
- 20 preferably, the humanized anti-IL-5 antibody comprises:
- a) the heavy chain variable region as shown in SEQ ID NO: 51 and the light chain variable region as shown in SEQ ID NO: 47;
- b) the heavy chain variable region as shown in SEQ ID NO: 65 and the light chain variable region as shown in SEQ ID NO: 62;
- 25 c) the heavy chain variable region as shown in SEQ ID NO: 58 and the light chain variable region as shown in SEQ ID NO: 56;
- d) the heavy chain variable region as shown in SEQ ID NO: 71 and the light chain variable region as shown in SEQ ID NO: 68; or
- e) the heavy chain variable region as shown in SEQ ID NO: 79 and the light chain variable region as shown in SEQ ID NO: 73.
- 30

20. The pharmaceutical composition according to any one of claims 1 to 19, wherein the anti-IL-5 antibody comprises human antibody constant region(s); preferably comprises:
a human antibody heavy chain constant region as shown in SEQ ID NO: 52 and a human antibody light chain constant region as shown in SEQ ID NO: 53.

5

21. The pharmaceutical composition according to claim 20, wherein the anti-IL-5 antibody comprises:

- i) a heavy chain as shown in SEQ ID NO: 83 and a light chain as shown in SEQ ID NO: 84;
- ii) a heavy chain as shown in SEQ ID NO: 85 and a light chain as shown in SEQ ID NO: 86;
- 10 iii) a heavy chain as shown in SEQ ID NO: 87 and a light chain as shown in SEQ ID NO: 88;
- iv) a heavy chain as shown in SEQ ID NO: 89 and a light chain as shown in SEQ ID NO: 90; or
- v) a heavy chain as shown in SEQ ID NO: 91 and a light chain as shown in SEQ ID NO: 92.

22. The pharmaceutical composition according to any one of claims 1 to 21, wherein the
15 anti-IL-5 antibody is a monoclonal antibody or antigen-binding fragment thereof that competes for binding to human IL-5 with the anti-IL-5 antibody or the antigen-binding fragment thereof according to any one of claims 1 to 21.

23. A method for preparing the pharmaceutical composition according to any one of claims 1 to
20 22, comprising a step of replacing the stock solution of the anti-IL-5 antibody or the antigen-binding fragment thereof with a buffer, wherein the buffer is preferably acetic acid-sodium acetate buffer.

24. A lyophilized formulation comprising the anti-IL-5 antibody or the antigen-binding fragment
25 thereof, which is obtained by lyophilization of the pharmaceutical composition according to any one of claims 1 to 22; preferably, the lyophilization comprises the steps of pre-freezing, primary drying and secondary drying, successively.

25. A reconstituted solution comprising the anti-IL-5 antibody or the antigen-binding fragment
30 thereof, wherein the reconstituted solution is obtained by reconstitution of the lyophilized formulation according to claim 24.

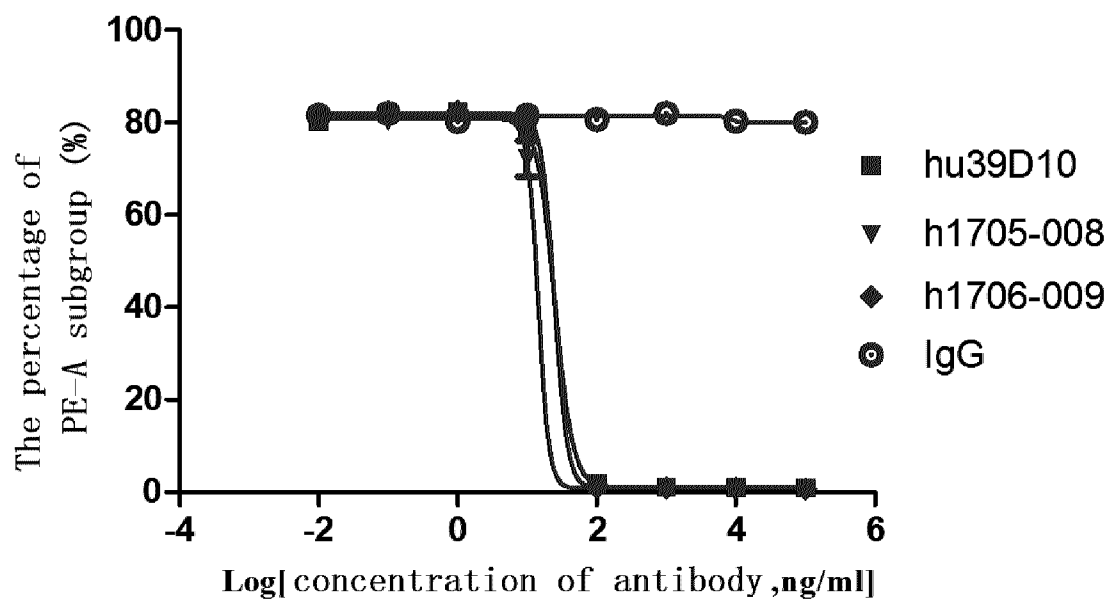
26. An article comprising a container, wherein the container comprises the pharmaceutical composition according to any one of claims 1 to 22, or the lyophilized formulation according to claim 24, or the reconstituted solution according to claim 25.

5

27. A method for treating IL-5 mediated disease, comprising administering a therapeutically effective amount of the pharmaceutical composition according to any one of claims 1 to 22, or the lyophilized formulation according to claim 24, or the reconstituted solution according to claim 25, or the article according to claim 26, to a subject in need thereof; wherein the IL-5 mediated disease is preferably selected from the group consisting of asthma, chronic pneumonia, allergic rhinitis, allergic bronchopulmonary aspergillosis disease, eosinophilia, Churg-Strauss syndrome, atopic dermatitis, onchocerciasis dermatitis, intermittent angioedema, eosinophilic myalgia syndrome, eosinophilic gastroenteritis, worm infection, Hodgkin's disease, nasal polyps, Loeffler's syndrome, urticaria, hypereosinophilic bronchitis, nodular arteritis, sinusitis, eosinophilic esophagitis, allergic eosinophilic esophagitis, allergic conjunctivitis, onchocerciasis dermatitis, endometriosis and steroid-dependent eosinophilic bronchitis.

10

15

**Figure 1**

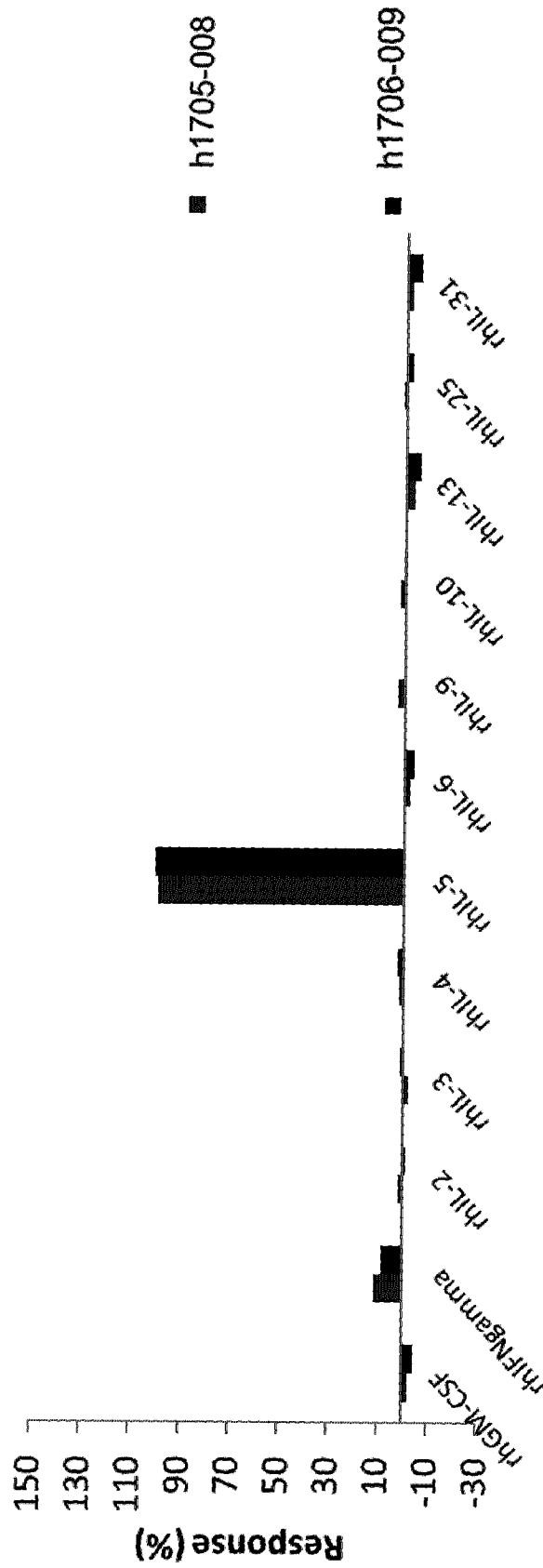


Figure 2

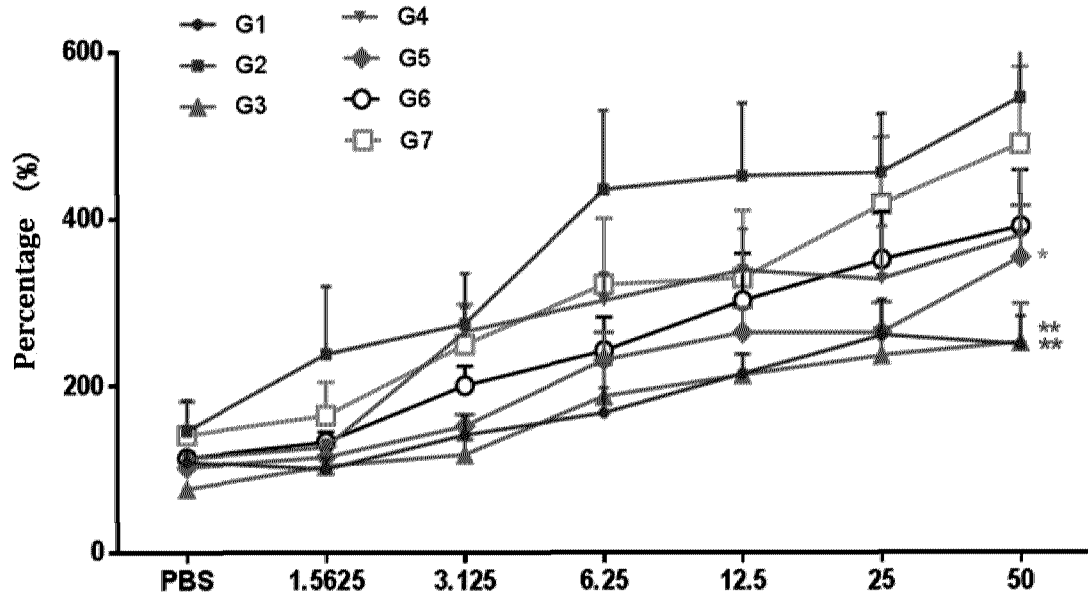


Figure 3

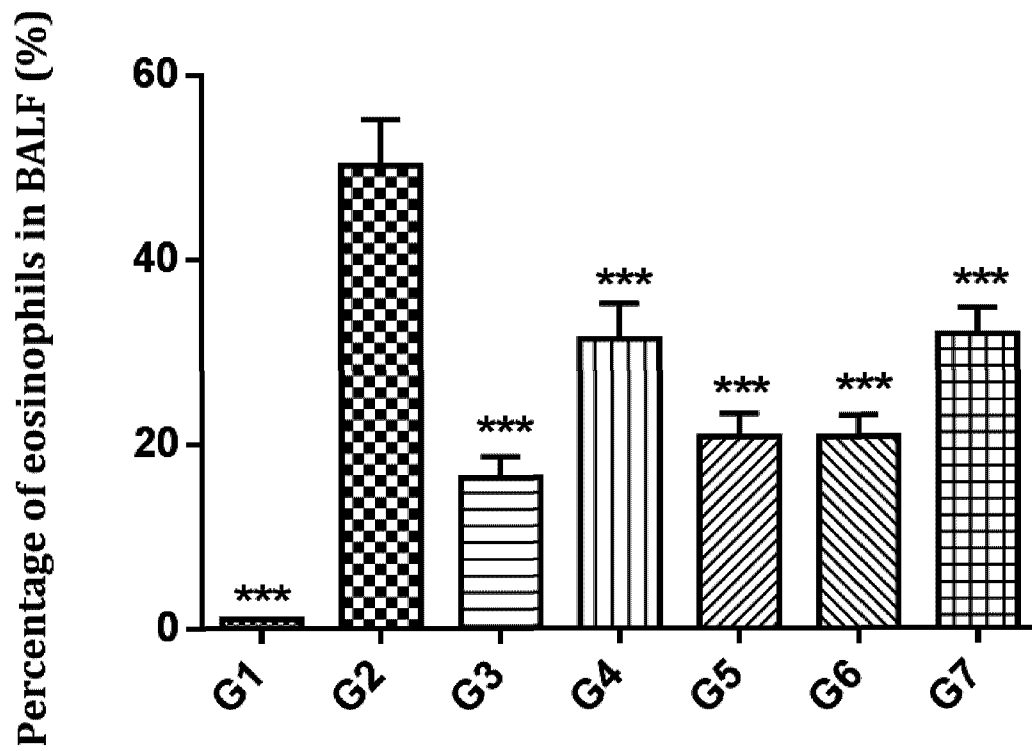
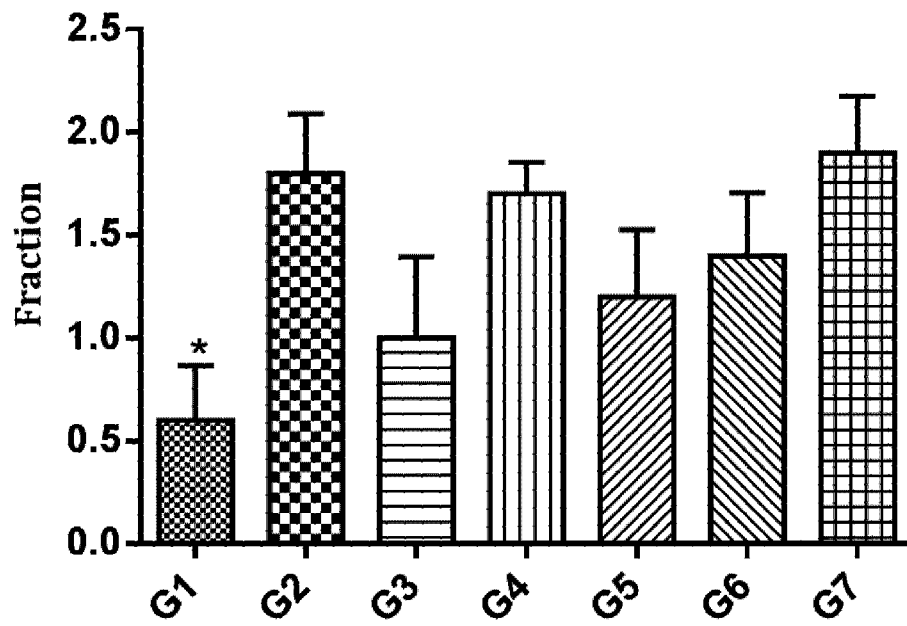
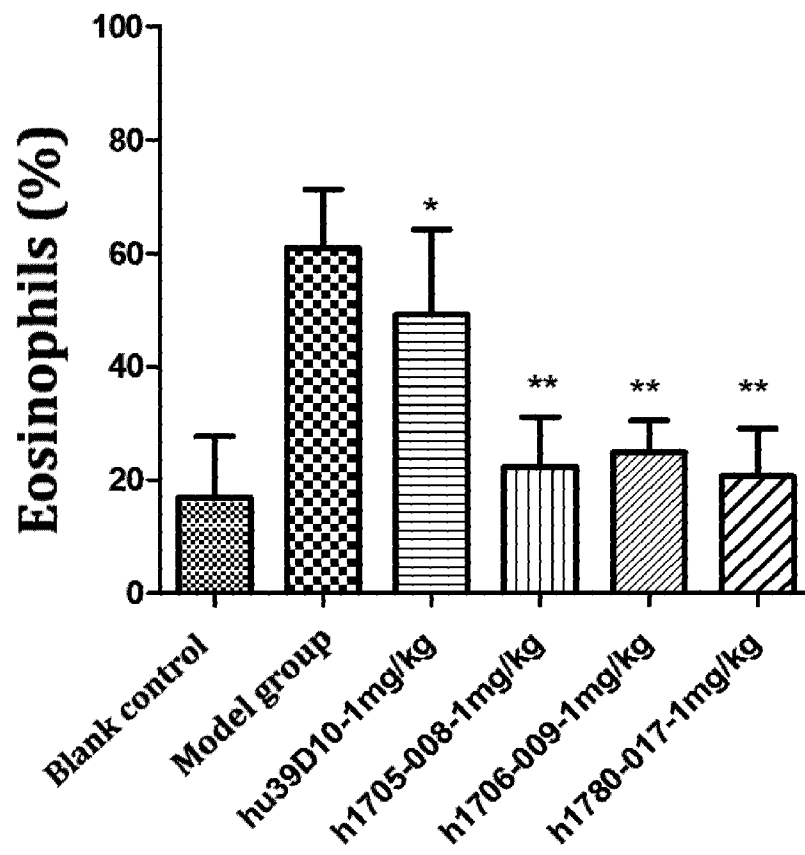
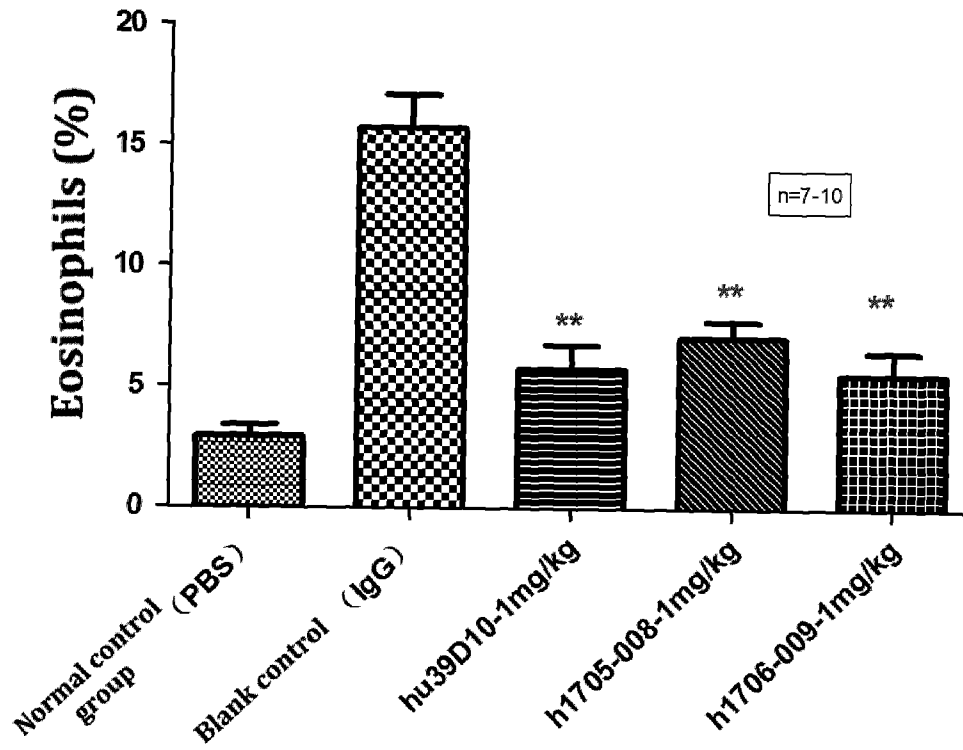
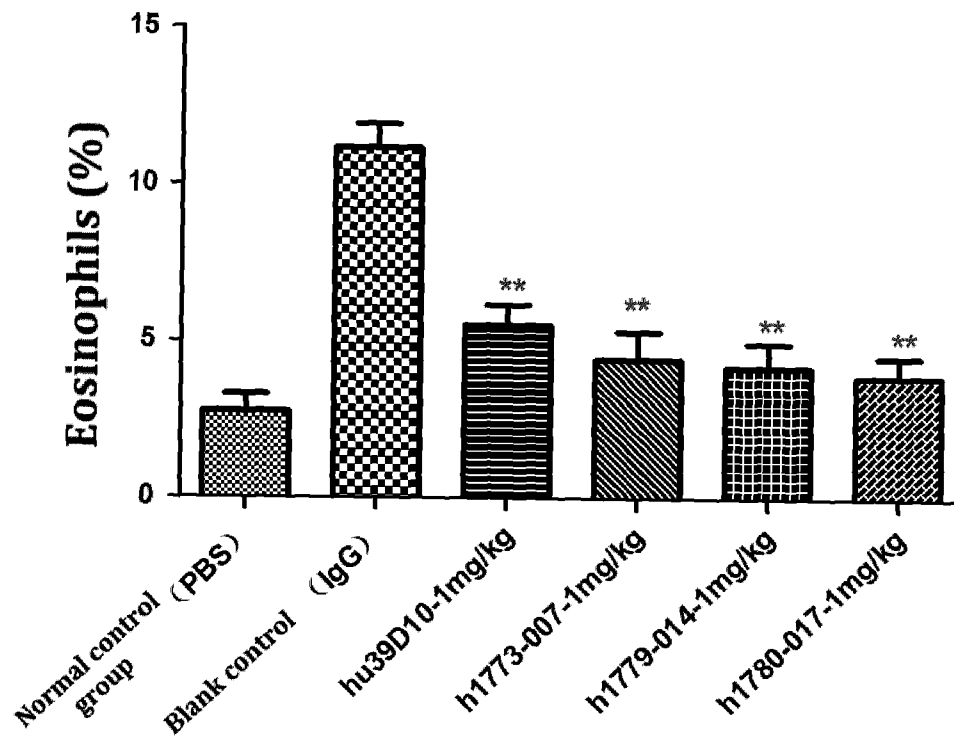


Figure 4A

**Figure 4B****Figure 4C**

**Figure 5A****Figure 5B**