What is claimed

1. A method for preparing a composition of antibody-drug conjugates (ADCs), comprising the following steps:

(a) reduction step: incubating a reductant and an antibody in a buffer system to reduce inter-chain disulfide bonds within the antibody to generate reduced thiol groups on a reduced antibody, optionally purifying the reduced antibody;

(b) re-oxidation step: adding an oxidant to re-oxidize some of the reduced thiol groups to generate a re-oxidized antibody, optionally purifying the re-oxidized antibody;

wherein the step (a) or the step (b) is performed in presence of a transition metal ion,

wherein in step (b), the reduced thiol groups which are not blocked by the transition metal ion are re-oxidated;

(c) conjugation step: removing the transition metal ion and adding an excess amount of a payload comprising a reactive group to react with reduced thiol groups of the re-oxidized antibody; and

(d) recovering step: recovering the resultant ADCs to obtain a composition of ADCs.

2. The method of claim 1, wherein the transition metal ion is selected from a group consisting of Zn^{2+}, Mn^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, and Cu^{2+}, preferably the transition metal ion is Zn^{2+}.

3. The method of claim 1 or 2, wherein the buffer system used in step (a) is selected from a group consisting of TAPS, Bicine, Tris, Tricine, HEPES, TES, MOPS, PIPES, Sodium citrate, Histidine buffer, PB (phosphate buffer), PBS, and MES, and wherein the buffer system has a pH value of 5 to 8, preferably about 7.0.

4. The method of any one of claims 1 to 3, wherein in step (a), the reductant is added at a molar ratio of 1 to 20 relative to the antibody.

5. The method of any one of claims 1 to 4, wherein the transition metal ion is added at a molar ratio of 0.5 to 50 relative to the antibody.

6. The method of any one of claims 1 to 5, wherein the reductant in step (a) is selected from a group consisting of Tris(2-carboxyethyl) phosphine (TCEP), Tris(3-hydroxypropyl)phosphine (THPP), 2-diphenylphosphanylbenzenesulfonic acid (diPPBs), Dithiothreitol (DTT), 2-(Diphenylphosphino) acetic acid (DPAA), Dithioerythritol (DTE), β-Mercaptoethanol, $LiAlH_4$, $Na_2S_2O_3$, KBH_4, and Hydrazine.

7. The method of any one of claims 1 to 6, wherein the oxidant in step (b) is selected from a group consisting of Hydrogen peroxide (H_2O_2), nitrate compounds, Potassium chlorate ($KClO_3$), Peroxydisulfuric acid ($H_2S_2O_8$), Peroxymonosulfuric acid (H_2SO_5), NaClO, Sodium dichromate ($Na_2Cr_2O_7$), Permanganate compounds, Sodium perborate, Nitrous oxide, Sodium bismuthate ($NaBiO_3$), ceric sulfate, dehydroascorbic acid (DHAA), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), and 2-Aminophenyl disulfide (DDD), preferably the oxidant in step (b) is DHAA or DTNB; and

wherein optionally in step (b), the oxidant is added at a molar ratio of 0.5 to 160 relative to the antibody.

8. The method of any one of claims 1 to 7, wherein in step (c), the transition metal ion is removed by adjusting pH, by adjusting temperature, by changing buffer, or by adding a metal chelator, and wherein the metal chelator is selected from a group consisting of EDTA, DTPA, Ethylenediamine, 2,2'-Bipyridine, and 1,10-phenanthroline.

9. The method of any one of claims 1 to 8, wherein the payload comprises a reactive group selected from a group consisting of maleimide moiety, bromide, iodide, electron deficient alkenyl or alkynyl, mono- or di- derivative of disulfides, sulfone,

bicyclo[1.1.0]butane derivative, sulfonyl fluoride, pentafluorophenol ester, palladium oxidative-addition complex, iodoxolone, and highly electron deficient arene, and/or the payload is selected from a group consisting of a diagnostic agent, a therapeutic agent and a labelling agent.

10. The method of any one of claims 1 to 9, wherein the antibody is a monoclonal antibody or a polyclonal antibody, or the antibody is a monospecific antibody or a multi-specific antibody, for example, a bispecific antibody,

preferably, wherein the antibody is a human antibody, a humanized antibody, a chimeric antibody or an antigen-binding moiety thereof.

11. The method of any one of claims 1 to 10, wherein the composition comprises D2 in a content higher than 64 mol%, preferably higher than 75 mol% or even higher than 80mol%, on the basis of total molar of D0, D2, D4, D6 and D8, or wherein the composition comprises D0, D4, D6 and D8 in a total content less than 36mol%, preferably less than 25mol%, more preferably less than 20 mol%, most preferably less than 15 mol%, on the basis of total molar of D0, D2, D4, D6 and D8,

wherein D0 represents an antibody molecule which is not conjugated with any drug,

D2 represents an ADC molecule in which two drug molecules are coupled to one single antibody molecule,

D4 represents an ADC molecule in which four drug molecules are coupled to one single antibody molecule,

D6 represents an ADC molecule in which six drug molecules are coupled to one single antibody molecule, and

D8 represents an ADC molecule in which eight drug molecules are coupled to one single antibody molecule.

12. A composition of antibody-drug conjugates (ADCs) prepared by the method of any one of claims 1 to 11, wherein the composition comprises D2 in a content higher

than 64 mol%, preferably higher than 75 mol% or even higher than 80mol%, on the basis of total molar of D0, D2, D4, D6 and D8, or wherein the composition comprises D0, D4, D6 and D8 in a total content less than 36mol%, preferably less than 25mol%, more preferably less than 20 mol%, most preferably less than 15 mol%, on the basis of total molar of D0, D2, D4, D6 and D8.

13. A pharmaceutical composition comprising an effective amount of the composition according to claim 12 and a pharmaceutically acceptable carrier.

14. Use of the composition according to claim 12 or the pharmaceutical composition according to claim 13 in the manufacture of a medicament for treating a condition or disorder in a subject, wherein a therapeutically effective amount of the composition according to claim 12 or the pharmaceutical composition according to claim 13 is to be administered to the subject.

15. The use of claim 14, wherein the condition or disorder is selected from a group consisting of a tumor, a cancer, an autoimmune disease, and an infectious disease, e.g., viral or microbial infection, and/or wherein the subject is a mammal, preferably a human.