

L234A, L235A and G237A.

In the present invention, letters before the position number represent amino acids before mutation, and letters after the position number represent amino acids after mutation, unless otherwise specified.

In one or more embodiments of the present invention, for the bispecific antibody, according to the EU numbering system, the heavy chain constant region of the immunoglobulin has one or more mutations selected from:

N297A, D265A, D270A, P238D, L328E, E233D, H268D, P271G, A330R, C226S, C229S, E233P, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D, G236R, G236A, L328R, A330S, P331S, H268A, E318A and K320A.

In a specific embodiment, the anti-CD73/anti-PD-1 bispecific antibody has a structure shown as heavy chain-light chain-linker 1-scFv, and the scFv is selected from 14C12H1V-linker 2-14C12L1V, 14C12H1V-linker 1-14C12L1V, 14C12H1V-linker 2-14C12L1V and 14C12H1V-linker 1-14C12L1V, particularly selected from the group consisting of:

(1) NTPDV1, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 85, the light chain has an amino acid sequence set forth in SEQ ID NO: 28, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the linker 2 has an amino acid sequence set forth in SEQ ID NO: 81, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64;

(2) NTPDV2, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 83, the light chain has an amino acid sequence set forth in SEQ ID NO: 28, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64;

(3) NTPDV3, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 85, the light chain has an amino acid sequence set forth in SEQ

ID NO: 96, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the linker 2 has an amino acid sequence set forth in SEQ ID NO: 81, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64; and

(4) NTPDV4, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 83, the light chain has an amino acid sequence set forth in SEQ ID NO: 96, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64.

In one embodiment of the present invention, the bispecific antibody binds to CD73 protein and /or PD-1 protein with a K_D of less than about 10^{-5} M, e.g., less than about 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M or less.

Yet another aspect of the present invention relates to a vector comprising the isolated nucleic acid molecule disclosed herein.

Yet another aspect of the present invention relates to a host cell comprising the isolated nucleic acid molecule or the vector disclosed herein.

Yet another aspect of the present invention relates to a method for preparing the bispecific antibody disclosed herein, comprising culturing the host cell disclosed herein in a suitable condition and isolating the bispecific antibody from the cell cultures.

Yet another aspect of the present invention relates to a conjugate comprising a bispecific antibody and a conjugated moiety, wherein the bispecific antibody is the bispecific antibody disclosed herein and the conjugated moiety is a detectable label; specifically, the conjugated moiety is a radioisotope, a fluorescent substance, a chemiluminescent substance, a colored substance or an enzyme.

Yet another aspect of the present invention relates to a kit comprising the bispecific antibody disclosed herein or comprising the conjugate disclosed herein; preferably, the kit further comprises a secondary antibody that

light chain 19F3L3 of the immunoglobulin moiety in NTPDV1 or NTPDV2 is SEQ ID NO: 27. The amino acid sequence of the light chain 19F3L2 of the immunoglobulin moiety in NTPDV3 or NTPDV4 is SEQ ID NO: 96, and the nucleotide sequence of the light chain 19F3L2 of the immunoglobulin moiety
5 in NTPDV3 or NTPDV4 is SEQ ID NO: 100.

(1) NTPDV1, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 85, the light chain has an amino acid sequence set forth in SEQ ID NO: 28, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the
10 linker 2 has an amino acid sequence set forth in SEQ ID NO: 81, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64;

(2) NTPDV2, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 83, the light chain has an amino acid sequence set forth in SEQ ID NO: 28, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the
15 linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64;

(3) NTPDV3, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 85, the light chain has an amino acid sequence set forth in SEQ ID NO: 96, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the
20 linker 2 has an amino acid sequence set forth in SEQ ID NO: 81, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64; and

(4) NTPDV4, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 83, the light chain has an amino acid sequence set forth in SEQ ID NO: 96, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the
25 linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64.

CLAIMS

1. An anti-CD73/anti-PD-1 bispecific antibody comprising:
a first protein functional region targeting PD-1, and
5 a second protein functional region targeting CD73,
wherein
the first protein functional region comprises: HCDR1, HCDR2 and HCDR3
contained in a heavy chain variable region having an amino acid sequence set
forth in SEQ ID NO: 44; and
10 LCDR1, LCDR2 and LCDR3 contained in a light chain variable region having
an amino acid sequence set forth in SEQ ID NO: 49;
and the second protein functional region comprises: HCDR1, HCDR2 and
HCDR3 contained in a heavy chain variable region having an amino acid
sequence set forth in SEQ ID NO: 2; and
15 LCDR1, LCDR2 and LCDR3 contained in a light chain variable region having
an amino acid sequence set forth in SEQ ID NO: 7.

2. The anti-CD73/anti-PD-1 bispecific antibody according to claim 1,
wherein the first protein functional region comprises: HCDR1, HCDR2 and
HCDR3 set forth in SEQ ID NOs: 45–47 respectively, and LCDR1, LCDR2
20 and LCDR3 set forth in SEQ ID NOs: 50–52 respectively;
or the second protein functional region comprises: HCDR1, HCDR2 and
HCDR3 set forth in SEQ ID NOs: 3–5 respectively, and LCDR1, LCDR2 and
LCDR3 set forth in SEQ ID NOs: 8–10, respectively.

3. The anti-CD73/anti-PD-1 bispecific antibody according to claim 1 or 2,
25 wherein:
the first protein functional region comprises:
a sequence having an amino acid sequence set forth in SEQ ID NO: 44, or a
sequence having at least 80% sequence identity to the sequence set forth in
SEQ ID NO: 44; and
30 a sequence having an amino acid sequence set forth in SEQ ID NO: 49, or a

sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 49; or

(1) a sequence having an amino acid sequence set forth in SEQ ID NO: 62, or a sequence having at least 80% sequence identity to the sequence set forth in

5 SEQ ID NO: 62; and

a sequence having an amino acid sequence set forth in SEQ ID NO: 64, or a sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 64;

and/or,

10 the second protein functional region comprises

(1) a sequence having an amino acid sequence set forth in SEQ ID NO: 2, or a sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 2; and

a sequence having an amino acid sequence set forth in SEQ ID NO: 7, or a
15 sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 7;

or

(2) a sequence having an amino acid sequence set forth in SEQ ID NO: 20, or a sequence having at least 80% sequence identity to the sequence set forth in
20 SEQ ID NO: 20; and

a sequence having an amino acid sequence set forth in SEQ ID NO: 22, or a sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 22, or

(3) a sequence having an amino acid sequence set forth in SEQ ID NO: 20, or a
25 sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 20; and

a sequence having an amino acid sequence set forth in SEQ ID NO: 24, or a sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 24, or

30 (4) a sequence having an amino acid sequence set forth in SEQ ID NO: 93, or a

sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 93; and

a sequence having an amino acid sequence set forth in SEQ ID NO: 95, or a sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO: 95.

4. The anti-CD73/anti-PD-1 bispecific antibody according to claim 1 or 2, wherein: the numbers of the first protein functional region and the second protein functional region are independently 1, 2 or more.

5. The anti-CD73/anti-PD-1 bispecific antibody according to any one of claims 1–2, wherein: the first protein functional region and the second protein functional region are linked directly or via a linker.

6. The anti-CD73/anti-PD-1 bispecific antibody according to claim 5, wherein: the linker is (GGGGS) n , and n is a positive integer.

7. The anti-CD73/anti-PD-1 bispecific antibody according to claim 6, wherein: n is 1, 2, 3, 4, 5 or 6.

8. The anti-CD73/anti-PD-1 bispecific antibody according to any one of claims 1–4, wherein: the first protein functional region and the second protein functional region are independently an immunoglobulin or an antigen-binding fragment, wherein the antigen-binding fragment is Fab, F(ab') $_2$ or a single chain variable fragment.

9. The anti-CD73/anti-PD-1 bispecific antibody according to claim 8, wherein the first protein functional region is an immunoglobulin and the second protein functional region is an antigen-binding fragment; or the first protein functional region is an antigen-binding fragment and the second protein functional region is an immunoglobulin.

10. The anti-CD73/anti-PD-1 bispecific antibody according to claim 9, wherein: the N terminus of the heavy chain variable region of the antigen-binding fragment is linked directly or via a linker to the C terminus of CH1 of the immunoglobulin, and the N terminus of the light chain variable region of the antigen-binding fragment is linked directly or via a linker to the C

terminus of the light chain constant region CL of the immunoglobulin; or

the N terminus of the heavy chain variable region of the antigen-binding fragment is linked directly or via a linker to the C terminus of the light chain constant region CL of the immunoglobulin, and the N terminus of the light chain variable region of the antigen-binding fragment is linked directly or via a linker to the C terminus of the heavy chain constant region CH1 of the immunoglobulin, or

the C terminus of the heavy chain variable region of the antigen-binding fragment is linked directly or via a linker to the N terminus of the heavy chain of the immunoglobulin, and the C terminus of the light chain variable region of the antigen-binding fragment is linked directly or via a linker to the N terminus of the light chain of the immunoglobulin; or

the C terminus of the heavy chain variable region of the antigen-binding fragment is linked directly or via a linker to the N terminus of the light chain of the immunoglobulin, and the C terminus of the light chain variable region of the antigen-binding fragment is linked directly or via a linker to the N terminus of the heavy chain of the immunoglobulin.

11. The anti-CD73/anti-PD-1 bispecific antibody according to claim 10, wherein: the first protein functional region is an immunoglobulin and the second protein functional region is a single chain variable fragment; or the first protein functional region is a single chain variable fragment and the second protein functional region is an immunoglobulin.

12. The anti-CD73/anti-PD-1 bispecific antibody according to claim 11, wherein: the single chain variable fragment is a molecule formed by connecting a heavy chain variable region and a light chain variable region via a linker.

13. The anti-CD73/anti-PD-1 bispecific antibody according to claim 12, wherein the single chain variable fragment has the following structure:
NH₂-V_L-linker-V_H-COOH, linker-V_H-linker-V_L-COOH,
linker-V_L-linker-V_H-COOH, or NH₂-V_H-linker-V_L-COOH.

14. The anti-CD73/anti-PD-1 bispecific antibody according to claim 8, wherein: the antigen-binding fragment is a single chain variable fragment, when the single chain variable fragment is linked to the C terminus of the heavy chain of the immunoglobulin, the N terminus of the heavy chain of the immunoglobulin, or the C terminus of CH1 of the heavy chain constant region via a linker, a heavy chain variable region of the single chain variable fragment is firstly linked, or a light chain variable region of the single chain variable fragment is firstly linked.

15. The anti-CD73/anti-PD-1 bispecific antibody according to claim 14, wherein:

(1) a heavy chain variable region of the immunoglobulin comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 3–5, and a light chain variable region of the immunoglobulin comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 8–10; and

a heavy chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 45–47, and a light chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 50–52,

or

(2) a heavy chain variable region of the immunoglobulin comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 45–47, and a light chain variable region of the immunoglobulin comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 50–52; and,

a heavy chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 3–5, and a light chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 8–10.

16. The anti-CD73/anti-PD-1 bispecific antibody according to claim 15, wherein when the single chain variable fragment is linked to the C terminus of the heavy chain of the immunoglobulin via a linker, the heavy chain variable

region of the single chain variable fragment comprising CDRs having amino acid sequences set forth in SEQ ID NOs: 45–47 may be firstly linked, or the light chain variable region of the single chain variable fragment comprising CDRs having amino acid sequences set forth in SEQ ID NOs: 50–52 may be firstly linked,

or

wherein, when the single chain variable fragment is linked to the C terminus of the heavy chain of the immunoglobulin via a linker, the heavy chain variable region of the single chain variable fragment comprising CDRs having amino acid sequences set forth in SEQ ID NOs: 3–5 may be firstly linked, or the light chain variable region of the single chain variable fragment comprising CDRs having amino acid sequences set forth in SEQ ID NOs: 8–10 may be firstly linked.

17. The anti-CD73/anti-PD-1 bispecific antibody according to claim 8, wherein: the immunoglobulin is IgG, IgA, IgD, IgE or IgM.

18. The anti-CD73/anti-PD-1 bispecific antibody according to claim 11, wherein: one immunoglobulin molecule is linked to two single chain variable fragment molecules.

19. The anti-CD73/anti-PD-1 bispecific antibody according to claim 18, wherein the two single chain variable fragment molecules are identical.

20. The anti-CD73/anti-PD-1 bispecific antibody according to claim 14, wherein: a heavy chain variable region of the immunoglobulin comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 45–47, and a light chain variable region of the immunoglobulin comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 50–52;

and,

a heavy chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 3–5, and a light chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 8–10, when the single

chain variable fragment is linked to the C terminus of a heavy chain of the immunoglobulin via a linker, the heavy chain variable region of the single chain variable fragment comprising CDRs having amino acid sequences set forth in SEQ ID NOs: 3–5 may be firstly linked, or the light chain variable
5 region of the single chain variable fragment comprising CDRs having amino acid sequences set forth in SEQ ID NOs: 8–10 may be firstly linked.

21. The anti-CD73/anti-PD-1 bispecific antibody according to claim 14, wherein:

a heavy chain variable region of the immunoglobulin comprises CDRs having
10 amino acid sequences set forth in SEQ ID NOs: 3–5, and a light chain variable region of the immunoglobulin comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 8–10; and,

a heavy chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 45–47, and a
15 light chain variable region of the single chain variable fragment comprises CDRs having amino acid sequences set forth in SEQ ID NOs: 50–52,

wherein, when the single chain variable fragment is linked to the C terminus of a heavy chain of the immunoglobulin via a linker, the heavy chain variable region of the single chain variable fragment comprising CDRs having amino
20 acid sequences set forth in SEQ ID NOs: 45–47 may be firstly linked, or the light chain variable region of the single chain variable fragment comprising CDRs having amino acid sequences set forth in SEQ ID NOs: 50–52 may be firstly linked.

22. The anti-CD73/anti-PD-1 bispecific antibody according to claim 14,
25 wherein:

a heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 44, and a light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 49; or

a heavy chain variable region of the immunoglobulin has an amino acid
30 sequence set forth in SEQ ID NO: 62, and a light chain variable region of the

immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 64;
and/or,

(1) a heavy chain variable region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 2, and a light chain variable
5 region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 7; or

(2) a heavy chain variable region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 20, and a light chain variable
10 region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 22; or

(3) a heavy chain variable region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 20, and a light chain variable
region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 24; or

15 (4) a heavy chain variable region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 93, and a light chain variable region of the single chain variable fragment has an amino acid sequence set forth in SEQ ID NO: 95;

wherein, when the single chain variable fragment is linked to the C terminus of
20 a heavy chain of the immunoglobulin via a linker, the heavy chain variable region of the single chain variable fragment may be firstly linked, or the light chain variable region of the single chain variable fragment may be firstly linked.

23. The anti-CD73/anti-PD-1 bispecific antibody according to claim 14,
25 wherein: (1) a heavy chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 2, and a light chain variable region of the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 7; or

(2) a heavy chain variable region of the immunoglobulin has an amino
30 acid sequence set forth in SEQ ID NO: 20, and a light chain variable region of

the immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 22;
or

(3) a heavy chain variable region of the single chain variable fragment has
an amino acid sequence set forth in SEQ ID NO. 20, and a light chain variable
5 region of the single chain variable fragment has an amino acid sequence set
forth in SEQ ID NO. 24; or

(4) a heavy chain variable region of the immunoglobulin has an amino acid
sequence set forth in SEQ ID NO:93, and a light chain variable region of the
immunoglobulin has an amino acid sequence set forth in SEQ ID NO:
10 95;and/or,

(i) a heavy chain variable region of the single chain variable fragment has
an amino acid sequence set forth in SEQ ID NO: 44, and a light chain variable
region of the single chain variable fragment has an amino acid sequence set
forth in SEQ ID NO: 49, or

15 (ii) a heavy chain variable region of the single chain variable fragment has
an amino acid sequence set forth in SEQ ID NO: 62, and a light chain variable
region of the single chain variable fragment has an amino acid sequence set
forth in SEQ ID NO:64,

wherein, when the single chain variable fragment is linked to the C terminus of
20 a heavy chain of the immunoglobulin via a linker, the heavy chain variable
region of the single chain variable fragment may be firstly linked, or, the light
chain variable region may be linked firstly.

24. The anti-CD73/anti-PD-1 bispecific antibody according to claim 22 or
23, wherein: the immunoglobulin comprises a non-CDR region, and the
25 non-CDR region is derived from a non-murine species.

25. The anti-CD73/anti-PD-1 bispecific antibody according to claim 24,
wherein: a heavy chain constant region of the immunoglobulin is an Ig
gamma-1 chain C region, ACCESSION: P01857; and a light chain constant
region of the immunoglobulin is an Ig kappa chain C region, ACCESSION:
30 P01834, and/or

a heavy chain constant region of the immunoglobulin mutates at any 2 or 3 of positions 234, 235 and 237 based on Ig gamma-1 chain C region, ACCESSION: P01857, and after the mutation, the bispecific antibody has a reduced affinity constant to FcγRIa, FcγRIIIa and/or C1q compared with that
5 before the mutation.

26. The anti-CD73/anti-PD-1 bispecific antibody according to claim 25, wherein: according to the EU numbering system, the heavy chain constant region has the following mutations at positions 234, 235 and/or 237 based on Ig gamma-1 chain C region, ACCESSION: P01857:

10 L234A and L235A;

L234A and G237A;

L235A and G237A;

or

L234A, L235A and G237A,

15 or, the heavy chain constant region of the immunoglobulin also has one or more mutations selected from the following mutations:

N297A, D265A, D270A, P238D, L328E, E233D, H268D, P271G, A330R, C226S, C229S, E233P, P331S, S267E, L328F, A330L, M252Y, S254T, T256E, N297Q, P238S, P238A, A327Q, A327G, P329A, K322A, T394D,
20 G236R, G236A, L328R, A330S, P331S, H268A, E318A and K320A.

27. The anti-CD73/anti-PD-1 bispecific antibody according to claim 14, wherein the anti-CD73/anti-PD-1 bispecific antibody is selected from the group consisting of:

(i) P1D7V01, of which the heavy chain in immunoglobulin has an amino acid
25 sequence set forth in SEQ ID NO: 66, the light chain in immunoglobulin has an amino acid sequence set forth in SEQ ID NO:68, the linker 1 has an amino acid sequence set forth in SEQ ID NO:79, the scFv consists of the heavy chain variable region having an amino acid set forth in SEQ ID NO:20, linker 1 having an amino acid sequence set forth in SEQ ID NO:79, and the light chain
30 variable region having an amino acid set forth in SEQ ID NO:24,

- (ii) P1D7V02R, of which the heavy chain in immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 66, the light chain in immunoglobulin has an amino acid sequence set forth in SEQ ID NO:68, the linker 1 has an amino acid sequence set forth in SEQ ID NO:79, the scFv consists of the light chain variable region having an amino acid set forth in SEQ ID NO:24, linker 1 having an amino acid sequence set forth in SEQ ID NO:79, and the heavy chain variable region having an amino acid set forth in SEQ ID NO:20,
- (iii) P1D7V03, of which the heavy chain in immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 66, the light chain in immunoglobulin has an amino acid sequence set forth in SEQ ID NO:68, the linker 1 has an amino acid sequence set forth in SEQ ID NO:81, the scFv has the heavy chain variable region having an amino acid set forth in SEQ ID NO:20, linker 1 having an amino acid sequence set forth in SEQ ID NO:79, and the light chain variable region having an amino acid set forth in SEQ ID NO:24, and
- (iv) P1D7V04R, of which the heavy chain in immunoglobulin has an amino acid sequence set forth in SEQ ID NO: 66, the light chain in immunoglobulin has an amino acid sequence set forth in SEQ ID NO:68, the linker 1 has an amino acid sequence set forth in SEQ ID NO:81, the scFv consists of the light chain variable region having an amino acid set forth in SEQ ID NO:24, linker 1 having an amino acid sequence set forth in SEQ ID NO:79, and the heavy chain variable region having an amino acid set forth in SEQ ID NO:20,
- or the anti-CD73/anti-PD-1 bispecific antibody is selected from the group consisting of:
- (1) NTPDV1, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 85, the light chain has an amino acid sequence set forth in SEQ ID NO: 28, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the linker 2 has an amino acid sequence set forth in SEQ ID NO: 81, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64;
- (2) NTPDV2, of which the heavy chain has an amino acid sequence set forth in

SEQ ID NO: 83, the light chain has an amino acid sequence set forth in SEQ ID NO: 28, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, and
5 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64;
(3) NTPDV3, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 85, the light chain has an amino acid sequence set forth in SEQ ID NO: 96, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the
10 linker 2 has an amino acid sequence set forth in SEQ ID NO: 81, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64; and
(4) NTPDV4, of which the heavy chain has an amino acid sequence set forth in SEQ ID NO: 83, the light chain has an amino acid sequence set forth in SEQ ID NO: 96, the linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, 14C12H1V has an amino acid sequence set forth in SEQ ID NO: 62, the
15 linker 1 has an amino acid sequence set forth in SEQ ID NO: 79, and 14C12L1V has an amino acid sequence set forth in SEQ ID NO: 64.

28. The anti-CD73/anti-PD-1 bispecific antibody according to claim 1 or 2, wherein: the anti-CD73/anti-PD-1 bispecific antibody binds to CD73 protein
20 and /or PD-1 protein with a K_D of less than 10^{-5} M.

29. An isolated nucleic acid molecule comprising a nucleotide sequence capable of encoding the bispecific antibody according to any one of claims 1-28.

30. A vector comprising the isolated nucleic acid molecule according to
25 claim 29.

31. A host cell comprising the isolated nucleic acid molecule of claim 29.

32. A method for preparing the anti-CD73/anti-PD-1 bispecific antibody, comprising: culturing the host cell according to claim 31 in a suitable condition and isolating the bispecific antibody from the cell cultures.

30 33. A conjugate comprising the anti-CD73/anti-PD-1 bispecific antibody

according to any one of claims 1–28 and a conjugated moiety, wherein the conjugated moiety is a detectable label.

34. The conjugate according to claim 33, wherein the conjugated moiety is a radioisotope, a fluorescent substance, a chemiluminescent substance, a colored substance or an enzyme.

35. A kit comprising the anti-CD73/anti-PD-1 bispecific antibody according to any one of claims 1–28 or the conjugate according to claim 33 or 34.

36. The kit according to claim 35, wherein: the kit further comprises a secondary antibody that specifically recognizes the bispecific antibody.

37. The kit according to claim 36, wherein: the secondary antibody further comprises a detectable label, e.g., a radioisotope, a fluorescent substance, a chemiluminescent substance, a colored substance or an enzyme.

38. Use of the anti-CD73/anti-PD-1 bispecific antibody according to any one of claims 1–28 in preparing a kit for detecting the presence or level of CD73 and/or PD-1 in a sample.

39. A pharmaceutical composition comprising the anti-CD73/anti-PD-1 bispecific antibody according to any one of claims 1–28 or the conjugate according to claim 33 or 34.

40. Use of the anti-CD73/anti-PD-1 bispecific antibody according to any one of claims 1–28 or the conjugate according to claim 33 or 34 in the manufacture of a medicament selected from the group consisting of the followings:

- a medicament for treating a tumor,
- a medicament for diagnosing a tumor,
- a medicament for inhibiting the enzyme activity reaction of CD73;
- a medicament for blocking the binding of PD-1 to PD-L1,
- a medicament for down-regulating the activity or level of PD-1,
- a medicament for relieving the immunosuppression of PD-1 in an organism,

a medicament for elevating IL-2 expression in T lymphocytes, and
a medicament for elevating IFN- γ expression in T lymphocytes, or
in the manufacture of an agent for detecting the level of CD73 in a sample.

41. An *in vitro* method comprising: administering to a cell an effective
5 amount of the anti-CD73/anti-PD-1 bispecific antibody according to any one of
claims 1–28 or the conjugate according to claim 33 or 34.