**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

Anti-CD279 (PD-1) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D132-3	J110	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Human PD-1 (programmed death-1) is a 55 kDa member of the immunoglobulin superfamily that is induced in cells undergoing apoptosis. The PD-1 protein contains an immunoreceptor tyrosine-based inhibitory motif and is expressed predominantly on activated T and B lymphocytes. PD-1 plays a key role in peripheral tolerance and autoimmune disease and is thought to be involved in the maintenance of peripheral self-tolerance by serving as a negative regulator of immune responses. Two novel members of the B7 family have been identified as PD-1 ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC). Evidence reported to date suggests overlapping functions for these two PD-1 ligands and their constitutive expression on some normal tissues and up-regulation on activated antigen-presenting cells.

SOURCE: This antibody was purified from hybridoma (clone J110) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the Fc-fusion human PD-1 protein.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human PD-1 on Western blotting and Flow cytometry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10-20 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Transfectant	Not tested	Not tested
Reactivity on WB	+		

INTENDED USE:

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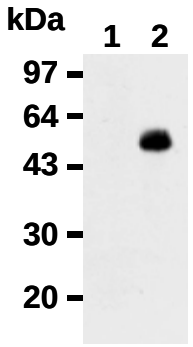
REFERENCES:

1) Bennett, F., *et al.*, *J. Immunol*. **170**, 711-719 (2003)
2) Iwai, Y., *et al.*, *Immunol. Lett*. **83**, 215-220 (2002)

Clone J110 is used in these references.

RELATED PRODUCTS:

D132-3 Anti-CD279 (PD-1) (Human) mAb (J110)
D132-4 Anti-CD279 (PD-1) (Human) mAb-FITC (J110)
D132-5 Anti-CD279 (PD-1) (Human) mAb-PE (J110)
D133-3 Anti-CD279 (PD-1) (Human) mAb (J105)
D133-5 Anti-CD279 (PD-1) (Human) mAb-PE (J105)
D092-3 Anti-CD274 (PD-L1) (Human) mAb (MIH3)
D092-6 Anti-CD274 (PD-L1) (Human) mAb-Biotin (MIH3)
D230-3 Anti-CD274 (PD-L1) (Human) mAb (27A2)
D231-3 Anti-CD273 (PD-L2) (Mouse) mAb (54-1)



Western blot analysis of PD-1 expression in X63 cells (1) and human PD-1 transfected X63 cells (2) using D132-3.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the

protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

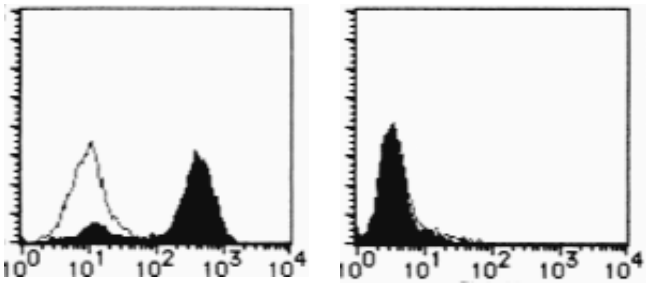
(Positive control for Western blotting; Transfectant)

Flow cytometric analysis for floating cells

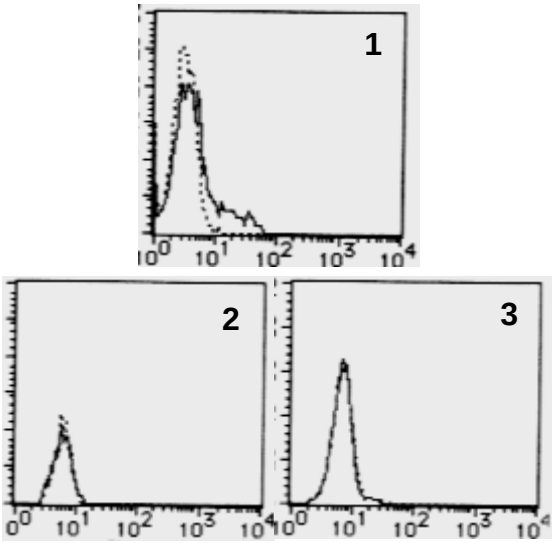
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 μL of the primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 30 μL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; Transfectant, peripheral blood lymphocyte)



Flow cytometric analysis of PD-1 expression on human PD-1 transfected X63 cells (left) and the parental cells (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D132-3 to the cells.*



Flow cytometric analysis of PD-1 expression on human peripheral blood lymphocytes (1), peripheral blood monocytes (2) and peripheral blood neutrophils (3). *Dot line histograms indicate the reaction of isotypic control to the cells. Solid line histograms indicate the reaction of D132-3 to the cells.*