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

MONOCLONAL ANTIBODY

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

Code No.	Clone	Subclass	Quantity	Concentration
D133-3	J105	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 (ITIM) 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 J105) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the human PD-1 Fc fusion 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 Flow cytometry.

APPLICATIONS:
Western blotting; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Immunoprecipitation; Not tested
Flow cytometry; 5 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Transfectant, Lymphocyte	Not tested	Not tested
Reactivity on FCM	+		

INTENDED USE:
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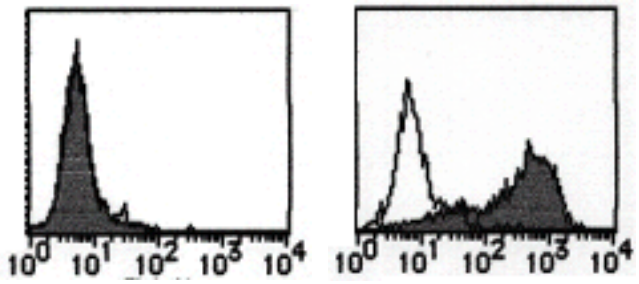
REFERENCES:
1) Kanai, T., *et al., J. Immunol.* **171,** 4156-4163 (2003)
2) Iwai, Y., *et al., Immunol. Lett.* **83**, 215-220 (2002)

Clone J105 is used in reference number 2).



Flow Cytometric analysis of PD-1 expression on X63 (left) and hPD-1/X63 (right). *Open histogram Indicates the reaction of isotypic control to the cells. Shaded Histograms indicate the reaction of D133-3 to the cells.*

PROTOCOLS:

Flow cytometric analysis for floating cells

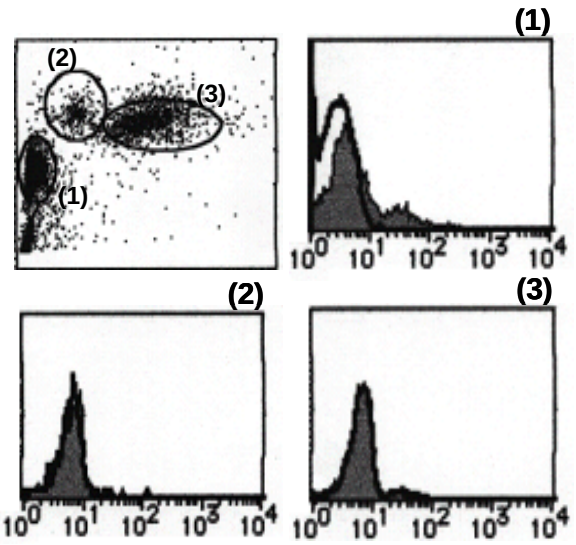
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

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 secondary antibody 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 control for flow cytometry; Transfectant)



Flow cytometric analysis of PD-1 expression on Human periferal blood Lymphocytes (1), Monocytes (2), and Granulocytes (3). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D133-3.*

Flow cytometric analysis for whole blood cells

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

1) Add 50 µL of the primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
3) Add 1 mL of PBS followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 50 µL of secondary antibody 1:20 Anti-IgG (Mouse) pAb-FITC (MBL; code no. IM-0819) diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

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)
D230-5	Anti-CD274 (PD-L1) (Human) mAb-PE (27A2)