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

MONOCLONAL ANTIBODY

FITC Labeled CD279/PD-1

Code No.	Clone	Subclass	Quantity	Concentration
D132-4	J110	Mouse IgG1	1 mL	50 µg/mL

BACKGROUND: Human PD-1 (programmed death-1) is a 55KDa 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 beads. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with a human recombinant CD279/PD-1.

FORMULATION: 50 µg IgG in 1 mL volume of PBS containing 1% BSA and 0.1% NaN_3.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with human CD279/PD-1.

APPLICATIONS:
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

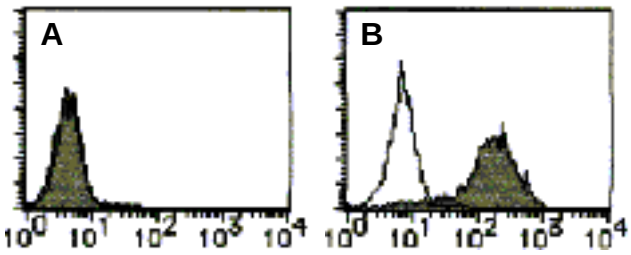
Species	Human	Mouse	Rat
Cells	Lymphocytes	Not tested	Not tested
Reactivity on FCM	+		

INTENDED USE:
For research use only. Not for clinical diagnosis.

REFERENCE:
1) Iwai, Y., *et al*., *Immunol. Lett*. **83**, 215-220 (2002)

Clone J110 is used in this reference.

RELATED PRODUCTS:
D132-3 CD279/PD-1 (J110)
D132-5 PE Labeled CD279/PD-1 (J110)
D133-3 CD279/PD-1 (J105)
D133-5 PE Labeled CD279/PD-1 (J105)
D092-3 CD274/PD-L1 (MIH3)
D092-6 Biotin labeled CD274/PD-L1 (MIH3)
D230-3 CD274/PD-L1 (27A2)
D230-5 PE Labeled CD279/PD-L1 (27A2)
MTG-001 Clear Back



Flow cytometric analysis of PD-1 expression on transfectant
A: Parental cell (X63)
B: Transfectant (human CD279/PD-1-X63)
■ D132-4
□ Isotype control

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 Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5

minutes at room temperature.
5) Add 30 μL of the FITC Labeled anti-Human PD-1 monoclonal antibody (J110) (10 μg/mL) 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) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for flow cytometry ; Lymphocytes)

Flow cytometric analysis for whole blood cells

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

1) Add 20 μL of the FITC Labeled anti-Human PD-1 monoclonal antibody (J110) (50 μg/mL) 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 2 mL of PBS followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) 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.
5) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.