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

MONOCLONAL ANTIBODY

PE labeled Anti-Human PD-1

Code No.	Clone	Subclass	Quantity
D133-5	J105	Mouse IgG1	50 tests

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 J105) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with a fusion protein of the extracellular domain of human PD-1 and the constant region of human γ1 heavy chain.

FORMULATION: 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% 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 PD-1 on Flow cytometry.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 20 µL (ready for use)

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

INTENDED USE:
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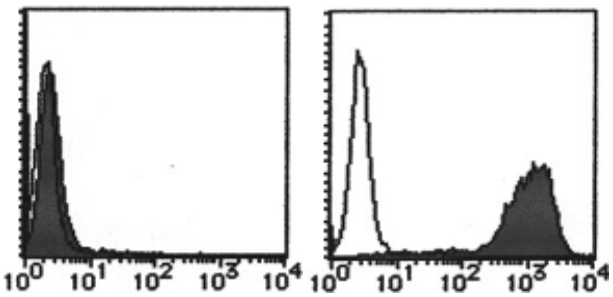
RELATED PRODUCTS:
D132-3 Anti-Human PD-1 (J110)
D132-4 FITC labeled anti-Human PD-1 (J110)
D132-5 PE labeled anti-Human PD-1 (J110)
D133-3 Anti-Human PD-1 (J105)
D092-6 Biotin labeled anti-B7-H1/PD-L1 (MIH3)

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Transfectant	Not Tested	Not Tested
Reactivity on FCM	+		

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 this reference number 2).



Flow cytometric analysis of PD-1 expression on X63 cells (left) and PD-1 transfected X63 cells (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D133-5 to the cells.*

PROTOCOL:
Flow cytometric analysis for floating cells
We usually use Fisher tubes or equivalents as reaction tubes for all step descrsibed 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 20 µL of PE labeled anti-PD-1 (J105). Mix well and incubate for 20 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; transfectant)