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

MONOCLONAL ANTIBODY

CD274/PD-L1

Code No.	Clone	Subclass	Quantity	Concentration
D092-3	MIH3	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: Programmed death ligand 1 (PD-L1, also known as CD274/B7-H1), a member of B7 family was identified by searching for molecules that share homology with the immunoglobulin V and C domains of B7-1 and B7-2 among the human cDNA expressed sequence tags in the National Center for Biotechnology Information database. PD-L1 is a ligand for programmed death 1 (PD-1) which belongs to the CD28/CTLA4 subfamily. Although *in vitro* study indicated that the cross-linking of PD-1 by PD-L1 leads to down-regulation of T-cell responses, some studies have shown that T cells stimulated with low levels of anti-CD3 and immobilized PD-L1-Ig were activated, proliferation and production of IFN-γ, GM-CSF and IL-10 from the T cells were enhanced.

SOURCE: This antibody was purified from hybridoma (clone MIH3) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with the full-length human PD-L1 transfected L cells.

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 CD274 antigen on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

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

INTENDED USE:

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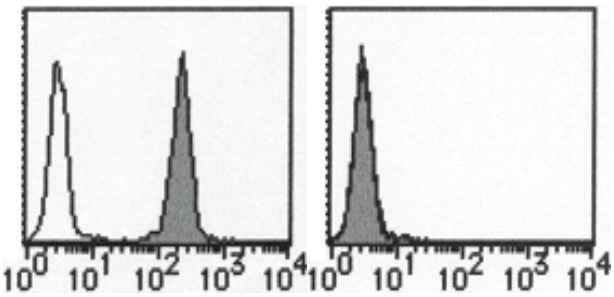
REFERENCES:

1) Yamazaki, T., *et al*., *J. Immunol*. **169**, 5538-5545 (2002)
2) Tamura, H., *et al*., *Blood* **97**, 1809-1816 (2001)
3) Dong, H., *et al*., *Nat. Med.* **12**, 1365-1369 (1999)



Flow cytometric analysis of CD274 expression on CD274 transfectant (left) and mock (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D092-3 to the cells.*

PROTOCOL:

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 suggest in the **APPLICATIONS** diluted in 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 FITC conjugated anti-mouse IgG (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.

RELATED PRODUCTS:

M073-3 Anti-Caspase-2 (4F8)
M097-3 Anti-Caspase-3 (1F3)
K0197-3 Anti-Caspase-3 (AMI-3-1-11)
M087-3 Anti-Caspase-3 (1F9)
M088-3 Anti-Caspase-3 (7D12)
M029-3 Anti-Caspase-4 (4B9)
M060-3 Anti-Caspase-5 (4F7)
M070-3 Anti-Caspase-6 (3E8)
M053-3 Anti-Caspase-7 (4G2)
M032-3 Anti-Caspase-8 (5F7)
M058-3 Anti-Caspase-8 (5D3)
M054-3 Anti-Caspase-9 (5B4)
M059-3 Anti-Caspase-10 (4C1)
K0206-3 Anti-Caspase-12 (14F7)
K0207-3 Anti-Caspase-12 (14F4)
K0193-3 Anti-Caspase-14 (8-1-71)
M010-3 Anti-Bax (4F11)
M028-3 Anti-Mouse TRAF1 (3D4)
M030-3 Anti-Bag-1 (4A2)
M031-3 Anti-TRADD (3E11)
M033-3 Anti-FADD (1F7)
M035-3 Anti-FADD (4G3)
M037-3 Anti-DFF45/ICAD (6B8)
M044-3 Anti-XIAP (2F1)
M056-3 Anti-RAIDD (4B12)
M072-3 Anti-BID (5C9)
M074-3 Anti-Apaf-1 (5C1)
M083-3 Anti-AcinusL (3H8)
M112-3 Anti-Mouse TRAF2 (6F8)
D026-3 Anti-Mouse Fas (CD95) (RMF2)
D027-3 Anti-Mouse Fas (CD95) (RMF6)
D038-3 Anti-Bcl-2 (83-8B)
D038-5 PE labeled Anti-Bcl-2 (83-8B)
D041-3 Anti-Human Fas ligand (4H9)
D041-4 FITC labeled Anti-Human Fas ligand (4H9)
D041-5 PE labeled Anti-Human Fas ligand (4H9)
D041-6 Biotin labeled Anti-Human Fas ligand (4H9)
D042-3 Anti-Human Fas ligand (4A5)
D057-3 Anti-Mouse Fas ligand (FLIM58)
D057-4 FITC labeled Anti-Mouse Fas ligand (FLIM58)
D057-6 Biotin labeled Anti-Mouse Fas ligand (FLIM58)
D069-3 Anti-Mouse Fas ligand (FLIM4)
D086-3 Anti-ASC (23-4)
D092-6 Biotin labeled Anti-PD-L1 (MIH3)
D230-3 CD274/PD-L1 (27A2)
D231-3 Mouse CD273/Mouse PD-L2 (54-1)
D132-3 Anti-PD-1 (J110)
D132-4 FITC labeled Anti-PD-1 (J110)
D133-3 Anti-PD-1 (J105)
D161-3 Anti-MFG-E8 (2422)
D199-3 Anti-MFG-E8 (18A2-G10)
D184-3 Anti-Granulysin (RB1)
D185-3 Anti-Granulysin (RC8)
D185-6 Biotin labeled Anti-Granulysin (RC8)
D186-3 Anti-Granulysin (RF10)
D200-3 Anti-Human BAFF/BLyS (1D6)
D200-4 FITC labeled Anti-Human BAFF/BLyS (1D6)
D201-3 Anti-Human BAFF-R/BR3 (8A7)
D201-4 FITC labeled Anti-Human BAFF-R/BR3 (8A7)
K0033-3 Anti-DR3 (B65)
K0033-4 FITC labeled Anti-DR3 (B65)
K0039-3 Anti-TNF-R1 (H398)
K0039-4 FITC labeled Anti-TNF-R1 (H398)
K0040-3 Anti-TNF-R2 (80M2)
K0040-4 FITC labeled Anti-TNF-R2 (80M2)
K0127-3 Anti-Daxx (DAXX-01)
K0145-3 Anti-CD30 (Ber-H2)
K0145-4 FITC labeled Anti-CD30 (Ber-H2)
K0151-3 Anti-Bax (5B7)
K0152-3 Anti-Bax (6A7)
K0153-3 Anti-Bcl-xL (2H12)
K0154-3 Anti-Bcl-2 (10C4)
K0157-3 Anti-IKKγ (I-κB Kinase γ) (DA10-12)
K0159-3 Anti-IKKγ (I-κB Kinase γ) (EA2-6)
K0194-3 Anti-HtrA2/Omi (18-1-83)
CM001-1 Anti-Cytochrome c (1E4)
PM004 Anti-Smac/DIABLO (Polyclonal)
PD005 Anti-Vimentin Fragment (V1) (Polyclonal)
PD006 Anti-SETβ (p41/p42) (Polyclonal)
PD007 Anti-SETβ (p42) (Polyclonal)
PD008 Anti-SETβ (p41) (Polyclonal)
591 Anti-Bad (Polyclonal)
592 Anti-Mouse TRAF2 (Polyclonal)
597 Anti-Mouse TRAF6 (Polyclonal)
4690 APOPCYTO Annexin V-Azami-Green Apoptosis Detection Kit
4700 MEBCYTO Apoptosis Kit
8445 MEBSTAIN Apoptosis TUNEL Kit Direct
8441 MEBSTAIN Apoptosis TUNEL Kit Ⅱ
4800 APOPCYTO Caspase-3 Colorimetric Assay Kit
4805 APOPCYTO Caspase-8 Colorimetric Assay Kit
4810 APOPCYTO Caspase-9 Colorimetric Assay Kit
4815 APOPCYTO Caspase-3 Fluorometric Assay Kit
4820 APOPCYTO Caspase-8 Fluorometric Assay Kit
4825 APOPCYTO Caspase-9 Fluorometric Assay Kit
4817 Intracellular Caspase-3 Activity Detection Kit
4822 Intracellular Caspase-8 Activity Detection Kit
4827 Intracellular Caspase-9Activity Detection Kit
4830 APOPCYTO Intracellular Caspases Activity Detection Kit