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

MONOCLONAL ANTIBODY

Anti-CD274 (PD-L1) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D230-3	27A2	Mouse IgG2b	100 µL	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 immunogloblin 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. The role of PD-L1 is now debatable.

SOURCE: This antibody was purified from hybridoma (clone 27A2) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Balb/c mouse splenocyte immunized with recombinant human PD-L1 extracellular domain.

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; 10 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 15 minutes each in 10 mM citrate buffer (pH 6.0)
Autoclave; 10 minutes at 120°C in 10 mM citrate buffer (pH 6.0)
* Recommended activation; Autoclave.
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

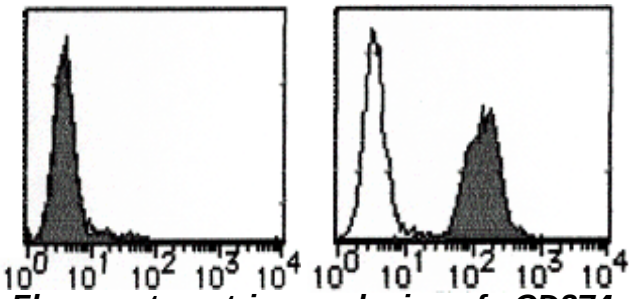
Species	Human	Mouse	Rat
Tissue	10 weeks placenta	Not tested	Not tested
Reactivity on IHC	+		

INTENDED USE:

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

REFERENCES:

1) Hamanishi, J., *et al., J. Clin. Oncol.* **33**, 4015-4022 (2015) [IHC]
2) Droeser, R. A., *et al., Eur. J. Cancer* **49**, 2233-2242 (2013) [IHC]
3) Gadiot, J., *et al.,* Cancer **117**, 2192-2201 (2011) [IHC]
4) Hino, R., *et al., Cancer* **116**, 1757-1766 (2010) [IHC]
5) Schuchmann, M., *et al., Am. J. Transplant.* **8**, 2434-2444 (2008) [IHC]
6) Hamanishi, J., *et al., PNAS.* **104**, 3360-3365 (2007) [IHC]
7) Trautmann, L., *et al., Nat. Med.* **12**, 1198-2202 (2006)
8) Day, C. L., *et al., Nature* **443**, 350-354 (2006)
9) Thompson, R. H., *et al., PNAS.* **101**, 17174-17179 (2004)



Flow cytometric analysis of CD274 expression on mock transfected p815 cells (left) and CD274 transfected p815 cells (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D230-3 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 0.09% 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.
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).

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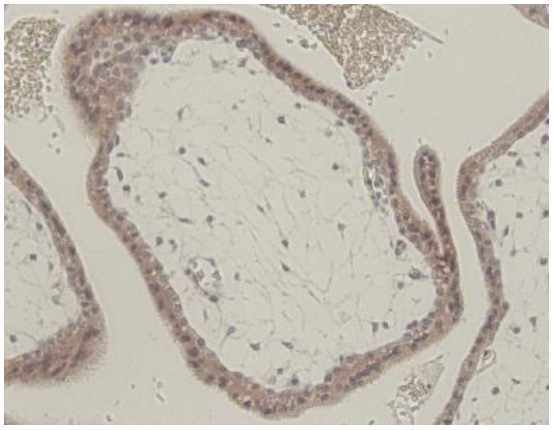
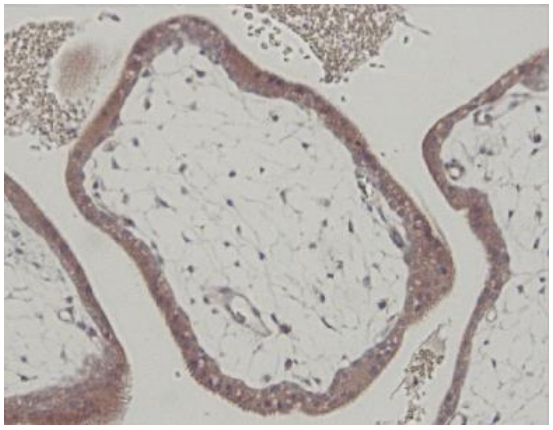
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

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 0.09% 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 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 FITC-conjugated anti-mouse IgG antibody 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.

activation; using Microwave



activation; using Autoclave



Immunohistochemical detection of CD274 on human 10 weeks placenta paraffin embedded section with D230-3.

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment

   Heat treatment by Microwave:

   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.0). Cover the beaker with plastic wrap, then process the slides 2 times for 15 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.

   Heat treatment by Autoclave:

   Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.0). Cover the beaker with plastic wrap, then process the slides with the autoclave for 10 minutes at 120°C. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 0.3% H_2O_2 in MeOH for 15 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 30 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
8) Incubate the sections overnight at 4°C.
9) Wash the slides 3 times in PBS-T [0.05% Tween-20 in PBS] for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 30 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 30 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 30 minutes with substrate solution (MBL; code no. IM-2394). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.

(Positive control for Immunohistochemistry; placenta)

RELATED PRODUCTS:

D230-5	Anti-CD274 (PD-L1) (Human) mAb-PE (27A2)
D092-3	Anti-CD274 (PD-L1) (Human) mAb (MIH3)
D092-3M2	Anti-CD274 (PD-L1) (Human) mAb (MIH3)
D092-6	Anti-CD274 (PD-L1) (Human) mAb-Biotin (MIH3)
D231-3	Anti-CD273 (PD-L2) (Mouse) mAb (54-1)
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)
D133-3M2	Anti-CD279 (PD-1) (Human) mAb (J105)
D133-5	Anti-CD279 (PD-1) (Human) mAb-PE (J105)
M073-3	Anti-Caspase-2 (Human) mAb (4F8)
M097-3	Anti-Caspase-3 (Human) mAb (1F3)
K0197-3	Anti-Caspase-3 (Human) mAb (AMI-3-1-11)
M087-3	Anti-Caspase-3 (Human) mAb (1F9)
M088-3	Anti-Caspase-3 (Human) mAb (7D12)
M029-3	Anti-Caspase-4 (Human) mAb (4B9)
M060-3	Anti-Caspase-5 mAb (4F7)
M070-3	Anti-Caspase-6 mAb (3E8)
M053-3	Anti-Caspase-7 mAb (4G2)
M032-3	Anti-Caspase-8 (Human) mAb (5F7)
M058-3	Anti-Caspase-8 (Human) mAb (5D3)
M054-3	Anti-Caspase-9 mAb (5B4)
M059-3	Anti-Caspase-10 (Human) mAb (4C1)
K0206-3	Anti-Caspase-12 mAb (14F7)
K0207-3	Anti-Caspase-12 mAb (14F4)
K0193-3	Anti-Caspase-14 (Human) mAb (8-1-71)
M010-3	Anti-BAX (Human) mAb (4F11)
M028-3	Anti-TRAF1 (Mouse) mAb (3D4)
M112-3	Anti-TRAF2 mAb (6F8)
592	Anti-TRAF2 pAb (Polyclonal)
M092-3	Anti-TRAF6 (Mouse) mAb (1F8)
597	Anti-TRAF6 (Mouse) pAb (Polyclonal)
CM002-1	Anti-TRAF6 mAb (1B1-2)
M030-3	Anti-Bag-1 mAb (4A2)
M033-3	Anti-FADD mAb (1F7)
M035-3	Anti-FADD (Human) mAb (4G3)
M037-3	Anti-DFF45 (ICAD) (Human) mAb (6B8)
M044-3	Anti-XIAP (MIHA/ILP-a) mAb (2F1)
M056-3	Anti-RAIDD (Human) mAb (4B12)
M072-3	Anti-BID (Human) mAb (5C9)
M074-3	Anti-Apaf-1 (Human) mAb (5C1)
M083-3	Anti-AcinusL (Human) mAb (3H8)
MD-10-3	Anti-Fas (CD95) (Human) mAb (UB2)
MD-11-3	Anti-Fas (CD95) (Human) mAb (ZB4)
D026-3	Anti-Fas (CD95) (Mouse) mAb (RMF2)
D027-3	Anti-Fas (CD95) (Mouse) mAb (RMF6)
SY-001	Anti-Fas (CD95) mAb (CH-11)
D041-3	Anti-Fas Ligand (CD178) (Human) mAb (4H9)
D042-3	Anti-Fas Ligand (CD178) (Human) mAb (4A5)
D057-3	Anti-Fas Ligand (CD178) (Mouse) mAb (FLIM58)
D069-3	Anti-Fas Ligand (CD178) (Mouse) mAb (FLIM4)
D038-3	Anti-Bcl-2 mAb (83-8B)
D038-5	Anti-Bcl-2 mAb-PE (83-8B)
D086-3	Anti-ASC (TMS1) (Human) mAb (23-4)
D161-3	Anti-MFG-E8 (Mouse) mAb (2422)
D199-3	Anti-MFG-E8 (Mouse) mAb (18A2-G10)
D184-3	Anti-Granulysin (Human) mAb (RB1)
D185-3	Anti-Granulysin (Human) mAb (RC8)
D185-6	Anti-Granulysin (Human) mAb-Biotin (RC8)
D186-3	Anti-Granulysin (Human) mAb (RF10)
D200-3	Anti-CD257 (BAFF/BLyS) (Human) mAb (1D6)
D200-4	Anti-CD257 (BAFF/BLyS) (Human) mAb -FITC (1D6)
D267-3	Anti-CD267 (TACI) (Human) mAb (11H3)
D267-5	Anti-CD267 (TACI) (Human) mAb-PE (11H3)
D201-3	Anti-CD268 (BAFF-R/BR3) (Human) mAb (8A7)
D201-4	Anti-CD268 (BAFF-R/BR3) (Human) mAb -FITC (8A7)
D201-5	Anti-CD268 (BAFF-R/BR3) (Human) mAb -PE (8A7)
K0039-3	Anti-CD120a (TNF-R1) (Human) mAb (H398)
K0040-3	Anti-CD120b (TNF-R2) (Human) mAb (80M2)
K0127-3	Anti-Daxx (Human) mAb (DAXX-01)
K0145-3	Anti-CD30 (Human) mAb (Ber-H2)
K0157-3	Anti-IKKγ (Human) mAb (DA10-12)
K0159-3	Anti-IKKγ mAb (EA2-6)
K0194-3	Anti-HtrA2 (Omi) (Human) mAb (18-1-83)
CM001-1	Anti-Cytochrome c mAb (1E4)
PM004	Anti-Smac (DIABLO) pAb (Polyclonal)
PD005	Anti-Vimentin Fragment (V1) pAb (Polyclonal)
PD006	Anti-SETβ (p41/p42) pAb (Polyclonal)
PD007	Anti-SETβ (p42) (Human) pAb (Polyclonal)
PD008	Anti-SETβ (p41) (Human) pAb (Polyclonal)
591	Anti-Bad pAb (Polyclonal)
4690	APOPCYTO Annexin V-Azami-Green Apoptosis Detection Kit
4700	MEBCYTO Apoptosis Kit
8445	MEBSTAIN Apoptosis TUNEL Kit Direct
8442	MEBSTAIN Apoptosis TUNEL Kit III
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

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