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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

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

Code No.	Clone	Subclass	Quantity
D230-5	27A2	Mouse IgG2b	1 mL (50 tests)

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. 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: 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 CD274 antigen on Flow cytometry.

APPLICATION:
Flow cytometry; 20 µL (ready for use)
*Please refer to the data sheet (MBL, code no. D230-3) for other applications.

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

SPECIES CROSS REACTIVITY:

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

INTENDED USE:
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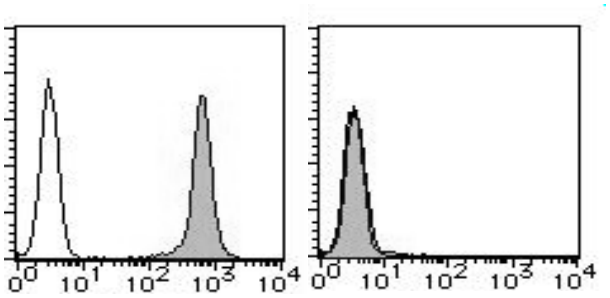
REFERENCES:
1) Hamanishi, J., *et al.*, *PNAS*, **104**, 3360-3365 (2007)
2) Trautmann, L., *et al.*, *Nat. Med.* **12**, 1198-2202 (2006)
3) Day, C. L., *et al.*, *Nature* **443**, 350-354 (2006)
4) Thompson, R. H., *et al.*, *PNAS.* **101**, 17174-17179 (2004)
5) Freeman, G. J., *et al.*, *J. Exp. Med.* **192**, 1027-1034 (2000)

Clone 27A2 is used in reference number 1).



Flow cytometric analysis of CD274 expression on CD274 transfected P815 cells (left) and mock transfectant (right). *Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D230-5 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 20 µL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 µL of the primary antibody at the concentration as suggested in the **APPLICATION** diluted with the washing buffer. 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.

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