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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Mouse CD300a/MAIR-I

Code No.	Clone	Subclass	Quantity	Concentration
D177-3	TX40	Rat IgG2a	100 µg	1 mg/mL

BACKGROUND: Immune responses are regulated by opposing positive and negative signals triggered by the interaction of activating and inhibitory cell surface receptors with their ligands. Shibuya *et al*. identified novel paired activated and inhibitory immunoglobulin-like receptors, designated myeloid-associated immunoglobulin-like receptor (MAIR) I and MAIR-II, whose extracellular domains are highly conserved by each other. MAIR-I, expressed on the majority of myeloid cells, including macrophages, granulocytes, mast cells, and dendritic cells, contains the tyrosine-based sorting motif and the immunoreceptor tyrosine-based inhibitory motif-like sequences in the cytoplasmic domains. On the other hand, MAIR-II, expressed on subsets of peritoneal macrophages and B cells, associates with the immunoreceptor tyrosine-based activation motif-bearing adaptor DAP12. MAIR-I is also known as CD300a/CMRF-35-like Ig-like molecule-8 (CLM-8)/leukocyte mono-Ig-like receptor 1 (LMIR1). MAIR-II is also known as CD300d/LMIR2/CLM-4/dendritic cell-derived Ig-like receptor 1 (DIgR1).

SOURCE: This antibody was purified from hybridoma (clone TX40) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0 with Wister rat lymphocyte immunized with the mouse MAIR-I transfected Ba/F3 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 -20ºC.

REACTIVITY: This antibody reacts with mouse CD300a 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 **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	WEHI-3B	Not Tested
Reactivity on FCM		+	

INTENDED USE:

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REFERENCES:

1) Nakahashi, C., *et al., J. Immunol*. **178**, 765-770 (2007)
2) Yotsumoto, K., *et al., J. Exp. Med*. **198**, 223-233 (2003)

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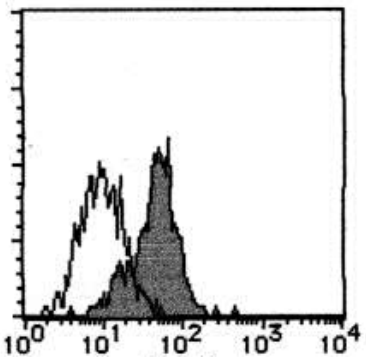

Flow cytometric analysis of mouse CD300a expression on WEHI-3B cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D177-3 to the cells.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps 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 (5 x 10^6 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 (20~25ºC). 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 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 PE conjugated anti-rat IgG (Beckman Coulter; code no. IM1623) 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.

(Positive control for Flow cytometry; WEHI-3B)

Flow cytometric analysis for whole blood cells

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

1) Add 50 μL of mouse CD300a monoclonal antibody (TX40) at the concentration as suggest in the **APPLICATIONS** diluted in the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3*] into each tube.
   *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) Add 50 μL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 ºC).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Add 30 μL of 1:100 PE conjugated anti-rat IgG Beckman Coulter; code no. IM1623) diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) 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.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.