

MONOCLONAL ANTIBODY

Mouse CD300a/d (MAIR-I/II)

Code No.	Clone	Subclass	Quantity	Concentration
D178-3	TX10	Rat IgG1	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 TX10) 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-II 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 -20°C.

REACTIVITY: This antibody reacts with mouse CD300a/d antigen on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested*

*It is reported that TX10 can be used in Western blotting in the reference number 3).

Immunoprecipitation; Not tested*

*It is reported that TX10 can be used in Immunoprecipitation in the reference number 2).

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:

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

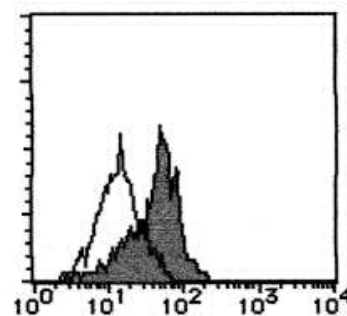
REFERENCES:

- 1) Nakahashi, C., *et al.*, *J. Immunol.* **178**, 765-770 (2007)
- 2) Okoshi, Y., *et al.*, *Int. Immunol.* **17**, 65-72 (2005)
- 3) Yotsumoto, K., *et al.*, *J. Exp. Med.* **198**, 223-233 (2003)

Clone TX10 is used in reference number 2) and 3).

RELATED PRODUCTS:

- D177-3 Mouse CD300a/MAIR-I (TX40)
- D177-4 FITC labeled Mouse CD300a/MAIR-I (TX40)
- D178-3 Mouse CD300a/d (MAIR-I/II) (TX10)
- D178-4 FITC labeled Mouse CD300a/d (MAIR-I/II) (TX10)
- D179-3 CD300d/MAIR-II (TX47)



Flow cytometric analysis of mouse CD300a/d expression on WEHI-3B cells. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D178-3 to the cells.

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 0.1% NaN₃].
- 2) Resuspend the cells with washing buffer (5 x 10⁶ 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% NaN₃ 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 (MBL; code no. IM-1623) 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/d monoclonal antibody (TX10) at the concentration as suggest in the **APPLICATIONS** diluted in the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃] into each tube.
- 2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25 °C).
- 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 (MBL; code no. IM-1623) 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 H₂O 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.