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AM26507AF-N Monoclonal Antibody to Mouse MAIR-2 (Myeloid-Associated Immunoglobulin-like Receptor) - Azide Free

Quantity:	0.1 mg
Concentration:	1.0 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).
Uniprot ID:	Q7TSN2
NCBI:	NP_598919.1
GeneID:	140497
Host / Isotype:	Mouse / IgG1
Clone:	TX45
Immunogen:	Ba/F3 transfectant expressing the human MAIR-II
Format:	**State:** Liquid Ig fraction **Purification:** Protein A agarose **Buffer System:** PBS containing 50% glycerol, pH 7.2. No preservative is contained.
Applications:	Flow cytometry: 10 µg/ml (final concentration). For details see protocols below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody reacts with CD300d antigen.
Species Reactivity:	**Tested:** Human (U937, Monocytes)
Add. Information:	This product was originally produced by MBL International.
Storage:	Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing. Shelf life: one year from despatch.

General Readings:

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).

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 10e6 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 20 µL of Clear Back (human Fc receptor blocking reagent) 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 20 µL of 1:40 FITC conjugated anti-mouse IgG 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; U937)

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 CD300d monoclonal antibody (TX47) 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 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 20 µL of 1:40 FITC conjugated anti-mouse IgG 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.

Pictures:

Flow cytometric analysis of CD300d in U937 (Left) and Jurkat (Right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of AM26507AF-N to the cells.