

**MONOCLONAL ANTIBODY**

# Anti-CD300C (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
W359-3	1E7D	Mouse IgG1 $\kappa$	100 $\mu$ L	1 mg/mL

**BACKGROUND:** CD300C is a member of the CD300 (also called CLM, LMIR, MAIR, IREM) family. The CD300 family of paired immune receptors consists of several activating and inhibitory receptors harboring a single immunoglobulin-like domain. Human CD300C is an activating receptor that is coupled to immunoreceptor tyrosin-based activating motif (ITAM)-containing Fc receptor  $\gamma$  (FcR $\gamma$ ). Human CD300C is mainly expressed in monocytes and mast cells.

**SOURCE:** This antibody was purified from hybridoma culture supernatant by Protein A affinity column chromatography.

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L 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 human CD300C on Flow cytometry.

**APPLICATIONS:**

- Flow cytometry; 1-10  $\mu$ g/mL
- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested

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

**INTENDED USE:**

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

**Entrez Gene ID:**

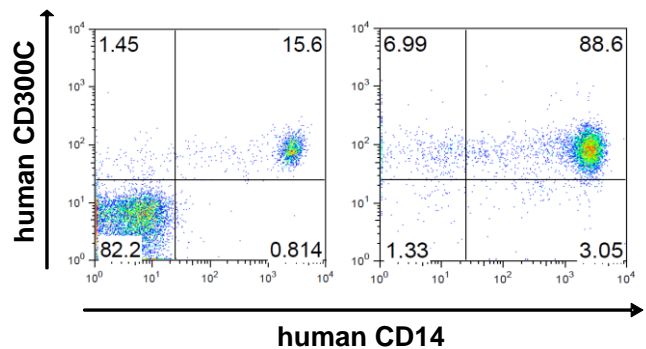
10871 (Human)

**REFERENCE:**

- 1) Takahashi M., et al., *J. Biol. Chem.* **288**, 7662-7675 (2013) [FCM]

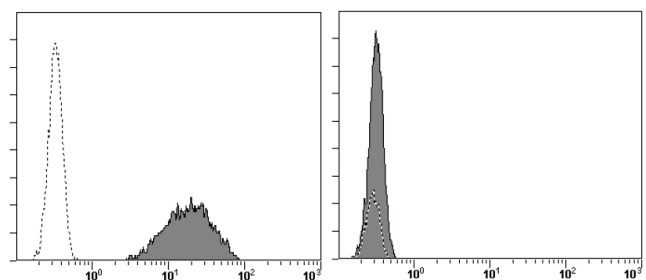
**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat	Hamster
Cells	Transfectant, Monocyte, PBMC	Not tested	Not tested	Not tested
Reactivity on FCM	+			



**Flow cytometric analysis of human CD300C expression on PBMC (left) and monocytes (right).**

The data were kindly provided by Dr. Jiro Kitaura, M.D. Ph.D. (Division of Cellular Therapy, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo)



**Flow cytometric analysis of human CD300C expression on CD300C (left) or CD300A (right) transfected Ba/F3. Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of W359-3 to the cells.**

## PROTOCOL:

### 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 0.05% NaN<sub>3</sub>].
- 2) Resuspend the cells with washing buffer (2.5 x 10<sup>6</sup> cells/mL).
- 3) Add 200 µL of cell suspension into each tube. And centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful decantation.
- 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 50 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 decantation.
- 7) Add 50 µL of 1:200 anti-mouse IgG-PE (Beckman Coulter; code no. IM0855) diluted with the washing buffer. Mix well and incubate for 30 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 decantation.
- 9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; transfectant)

## RELATED PRODUCTS:

W358-3	Anti-CD300A (Human) mAb
D177-3	Anti-CD300a (MAIR-I) (Mouse) mAb
D177-4	Anti-CD300a (MAIR-I) (Mouse) mAb-FITC
D178-3	Anti-CD300a/d (MAIR-I/II) (Mouse) mAb
D178-4	Anti-CD300a/d (MAIR-I/II) (Mouse) mAb-FITC
D179-3	Anti-CD300d (MAIR-II) (Human) mAb
M075-3	Mouse IgG1 (isotype control)
MTG-001	Clear Back (Human Fc receptor blocking reagent)