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

MONOCLONAL ANTIBODY

FITC labeled CD43

Code No.	Clone	Subclass	Quantity
D056-4	1D4	Mouse IgG2a	1 mL (50 tests)

BACKGROUND: CD43 antigen is a heavily sialylated single chain membrane glycoprotein with both molecular weight and antigenicity depending on the nature and complexity of the attached O-linked polysaccharides. CD43 is an integral membrane protein with extracellular domain (235 aa), transmembrane domain (23 aa) and intracytoplasmic domain (123 aa). The extracellular domain of CD43 has been shown to react with many ligand such as ICAM-1 (CD54), E-selectin, galectin-1, MHC-I and human serum albumin (HSA). The intracytoplasmic domain, which is highly conserved between species, is constitutively phosphorylated on serine residues. The level of phosphorylation increases after cell activation. This cell surface glycoprotein is also called leukosialin, sialophorin or GPL115. A soluble form of CD43 is also present in human serum. Thymocytes, $CD4^+$ T lymphocytes and monocytes express more of a 115 kDa CD43 isoform, whereas a 130 kDa isoform is found mostly on activated $CD4^+$ T cells, $CD8^+$ resting and activated T cells, neutrophils, platelets and B cells. CD43 plays a role in the regulation of lymphoid cell adhesion, cellular maturation and activation although the nature of this participation remains controversial. For example, the structural characteristics of the CD43 extracellular domain indicate that this molecule has both adhesive and anti-adhesive properties. O-glycosylated chains of CD43 such as a sialyl Lewis X (sLe^x) epitope, a ligand for both P- and E-selectin, potentiate binding to lectin-like molecules. However, the adhesion molecule function for CD43 is counteracted by the presence of negatively charged sialic acid residues on its extracellular domain. In addition to the strong structural evidence for the involvement of CD43 in cell interactions, experimental data suggest contradictive role for CD43 in regulating cell contacts.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 1D4) was established by fusion of mouse myeloma cell NS-1 with Balb/c mouse splenocyte immunized with PHA-activated human T cell line maintained with IL-2 containing media.

FORMULATION: 50 tests in 1 mL volume of NaPB (pH 8.0) containing 1% BSA, 150 mM NaCl 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 CD43 antigen on Flow cytometry. Clone 1D4 selectively recognizes core 2-containing *O*-glycans on CD43, which have the GlcNAcβ1→6 branch attached to the C-6 position of GalNAc and are formed by core 2 β-1,6-N-acetylglucosaminyltransferase (C2GnT). This type of CD43 is preferentially expressed in the $CD45RO^+$ memory subset of $CD4^+$ cells.

APPLICATION:

Flow cytometry; 20 µL (ready for use)

*Please refer to the data sheet (MBL code no. D056-3) for other applications.

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	lymphocyte, monocyte, granulocyte	Not Tested	Not Tested
Reactivity on FCM	+		

REFERENCES:

1) Weishaupt, C., *et al.*, *Clin. Cancer Res.* **13**, 2549-2556 (2007)
2) Hernandez, J. D., *et al.*, *J. Immunol.* **177**, 5328-5336 (2006)
3) Cabrera, P. V., *et al.*, *Blood* **108**, 2399-2406 (2006)
4) Fuhlbrigge, R. C., *et al.*, *Blood* **107**, 1421-1426 (2006)
5) Walcheck, B., *et al.*, *Blood* **99**, 4063-4069 (2002)
6) Harrington, L. E., *et al.*, *J. Exp. Med.* **191**, 1241-1246 (2000)
7) Mukasa, R., *et al.*, *Immunol. Lett.* **67**, 117-124 (1999)
8) Mukasa, R., *et al.*, *Int. Immunol.* **11**, 259-268 (1999)

Clone 1D4 is used in these references.

PROTOCOLS:

Flow cytometric analysis for whole blood cells

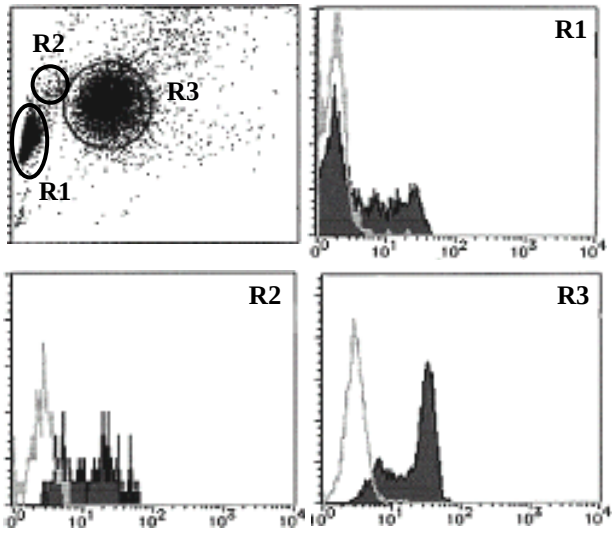
We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.

1) Add 20 µL of the primary antibody into each tube.
2) Add 100 µL of whole blood into each tube. Mix well, and incubate for 20 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) 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.
5) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
8) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; lymphocyte, monocyte, granulocyte)



Flow cytometric analysis of CD43 expression on lymphocyte (R1), monocyte (R2) and granulocyte (R3). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D056-4 to the cells.*

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% 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 into each tube. 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.

RELATED PRODUCTS:

D056-3	CD43 (1D4)
D056-5	PE labeled CD43 (1D4)
D119-3	mouse CD43 (MFT-3)
D064-3	CD4 (4H5)
K0003-1	CD4 (4B12)
K0006-1	CD8 (1A5)
K0015-3	CD45RO (UCHL1)
K0015-3	FITC labeled CD45RO (UCHL1)
M076-3	Mouse IgG2a Isotype control (6H3)
M076-4	FITC labeled Mouse IgG2a Isotype control (6H3)
M076-5	PE labeled Mouse IgG2a isotype control (6H3)