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

MONOCLONAL ANTIBODY

Mouse CD43

Code No.	Clone	Subclass	Quantity	Concentration
D119-3	MFT3	Rat IgG2a	100 µg	1 mg/mL

BACKGROUND: CD43 (also known as Ly48, leukosialin, and sialophorin) is a major cell surface sialoglycoprotein found on a variety of hematopoietically derived cells. It is a member of the surface mucin family, which plays a central role in cellular adhesion tumor progression. CD43 antigen is expressed on IL-7-responsive pro-B cell, plasmacyte, granulocyte, monocyte, macrophage, platelet, NK cell, peripheral cytotoxic T cell and majority of helper T cells. CD43 is not expressed on resting, conventional peripheral B cell. Two major glycoforms of mouse CD43 of 115 and 130 kDa have been identified. The 115-kDa glycoform is expressed on all T cells, whereas the 130-kDa glycoform is expressed predominantly on activated T cells. The 130-kDa glycoform of CD43 can function as a ligand for E-selectin on activated T cells and may potentially mediate activated T cell migration into sites of inflammation.

SOURCE: This antibody was purified from hybridoma (clone MFT3) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell SP2/0 with Wister rat splenocyte immunized with the autoreactive T-cell clones.

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 CD43 antigen (130 kDa glycoform only, but not 115 kDa glycoform) on Western blotting, Immunoprecipitation and Flow cytometry.

APPLICATIONS:

Western blotting; 5 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/250 µL of cell extract from 1 x 10^7 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10-20 µg/mL

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Not Tested	transfectant, IC2Tr	Not Tested
Reactivity on FCM		+	

INTENDED USE:

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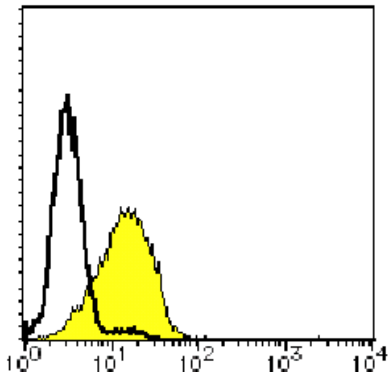
REFERENCE:

1) Fukuoka, M., *et al.*, *Blood* **96**, 4267-4275 (2000)

Clone MFT3 is used in this reference.

RELATED PRODUCTS:

D056-3 CD43 (1D4)
D056-4 FITC labeled CD43 (1D4)
D056-5 PE labeled CD43 (1D4)
K0004-1 CD5 (4C7)
K0009-1 CD23 (1B12)
D202-3 mouse CD11b (1C4)
D202-4 FITC labeled mouse CD11b (1C4)
M081-3 Rat IgG2a isotype control (2H3)



Flow cytometric analysis of mouse CD43 expression on IC2Tr cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D119-3 to the cells.*

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PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

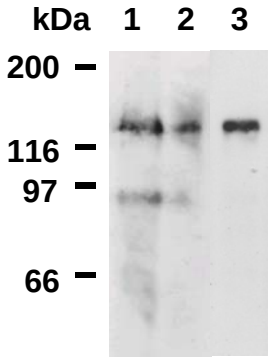
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 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 of 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 FITC conjugated anti-rat IgG (MBL; code no. 354) 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; IC2Tr)

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 CD43 monoclonal antibody (MFT3) as suggest in the **APPLICATIONS** diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] 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 FITC conjugated anti-rat IgG (MBL; code no. 354) 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.



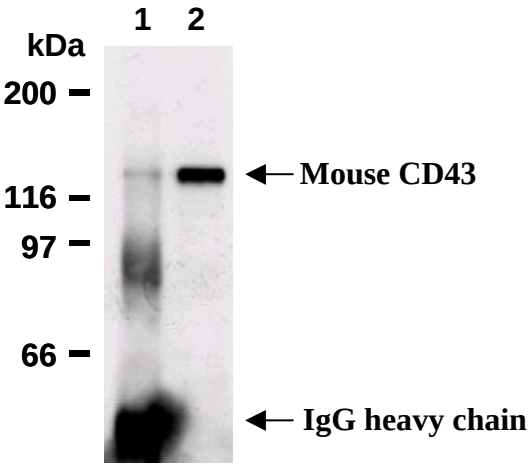
Western blot analysis of mouse CD43 expression in mouse bone marrow (1), mouse spleen (2) and IC2Tr (3) using D119-3.

SDS-PAGE & Western Blotting

1) Wash the $1x10^7$ cells 3 times with PBS and suspend with 1 mL of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 20 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with the 1:5,000 HRP-conjugated anti-rat IgG (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.

10) Expose to an X-ray film in a dark room for 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

    (Positive controls for Western blotting; mouse bone marrow, mouse spleen, IC2Tr)



Immunoprecipitation of mouse CD43 from IC2Tr cells with D119-3 (1). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D119-3. IC2Tr crude lysate was resolved in lane 2.*

Immunoprecipitation

1) Wash the cells 2 times with PBS and suspend with 500 µL of cold Lysis buffer (50 mM Tris-HCl pH 7.4, 250mM NaCl, 0.1% NP-40, 2mM EDTA) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add 50 µL of 50% protein G agarose beads in the supernatant. Incubate it at 4°C with rotating for 30 minutes.
4) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another two tube.
5) Add the rat IgG2a isotype control antibody (MBL; code no. M081-3) or mouse CD43 (MFT3) antibody at the amount of as suggest in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gently agitation for 60-120 minutes at 4°C.
6) Add 20 µL of 50% protein G agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
7) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
8) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µL/lane for the SDS-PAGE analysis.
   (See **SDS-PAGE & Western blotting**.)

   (Positive control for Immunoprecipitation; IC2Tr)