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

MBL
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MONOCLONAL ANTIBODY

Anti-CD11c (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M100-3	223H7	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: The CD11c (α X integrin; ~150 kDa) glycoprotein non-covalently associates with CD18 (β2 integrin; ~95 kDa) to form the heterodimeric complement receptor type 4 (CR4), which is involved in monocyte/granulocyte adhesion during inflammatory responses. The CD11c/CD18 receptor binds to CD54, iC3b and fibrinogen and plays a role in leukocyte adhesive interactions. CD11c/CD18 is also implicated in B cell proliferation and mediates B cell binding to fibrinogen. CD11c is commonly used as a marker for dendritic cells, but it is also expressed on macrophages, monocytes, granulocytes, NK cells, activated T and B lymphocytes and microglia.

SOURCE: This antibody was purified from hybridoma (clone 223H7) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Wister rat lymphocyte immunized with murine dendritic cells isolated from C57BL/6 mice.

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 CD11c antigen on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; 5 µg/1000 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested*
*It is reported that this antibody can be used in this application in the reference number 1), 2) and 4).
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)

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

INTENDED USE:

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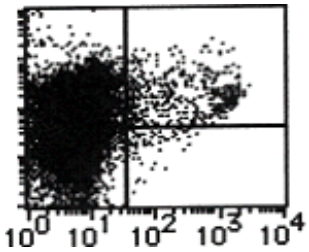
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not tested	Splenocyte	Not tested
Reactivity on FCM		+	

REFERENCES:

1) Catani, J. P., *et al., Transl. Oncol.* **9**, 565-574 (2016) [IHC]
2) Pilling, D. and Gomer, R. H., *PLoS One* **9**, e93730 (2014) [FCM, IHC]
3) Crawford, J. R., *et al. J. Leukoc. Biol.* **92**, 699-711 (2012) [FCM]
4) Ferreira, C. S., *et al., J. Virol.* **84**, 3033-3042 (2010) [IHC]
5) Kruger, T., *et al., J. Am. Soc. Nephrol.* **15**, 613-621 (2004)



Flow cytometric analysis of mouse CD11c expression on mouse splenocytes. *The staining intensity of M100-3 is shown in the horizontal axis with mouse I-A^b staining on the vertical axis.*

PROTOCOLS:

Flow cytometric analysis for floating cells

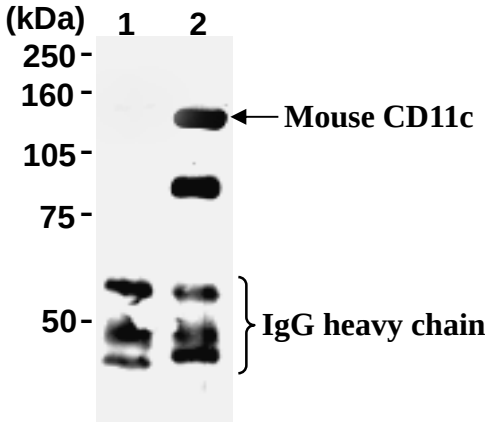
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% 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.
2) Resuspend the cells with washing buffer (5 x 10^6 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 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 30 µL of the primary antibody at the concentration

as suggested in the **APPLICATIONS** diluted with 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 FITC conjugated anti-rat IgG antibody 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) Add 30 µL of 1:50 Biotin conjugated anti-mouse I-A^b ($A^a\beta^b$) (BD Biosciences; code no. 553550) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) 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.
11) Add PE conjugated streptavidin diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
12) 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.
13) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse splenocyte)



Immunoprecipitation of mouse CD11c from biotin labeled JAWS II cells with rat IgG2a (1) or M100-3 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and detected with HRP-conjugated streptavidin.*

Immunoprecipitation

1) Wash the biotin labeled JAWS II cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] 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 the primary antibody as suggested in the **APPLICATIONS** into the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 30 µL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 2-4 times.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer and boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
9) 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% methanol). See the manufacture's manual for precise transfer procedure.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
11) Incubate the membrane with HRP-conjugated streptavidin diluted with 1% skimmed milk (in PBS, pH 7.2) for 15 minutes at room temperature.
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
13) 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.
14) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; JAWS II)

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