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

MBL

MONOCLONAL ANTIBODY

Biotin labeled Mouse CD11c

Code No.	Clone	Subclass	Quantity	Concentration
M100-6	223H7	Rat IgG2a	500 µg	0.5 mg/mL

BACKGROUND: The CD11c (integrin αX; ~150 kDa) glycoprotein non-covalently associates with CD18 (integrin β2; ~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 DC from C57BL/6 mice.

FORMULATION: 500 µg IgG in 1 mL volume of PBS containing 1% BSA 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 CD11c on Flow cytometry.

APPLICATIONS:
Cell separation; 20 µL/2.5x10^7 cells (ready for use)
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 2 µg/2.5x10^6 cells

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

RELATED PRODUCTS:
M100-3 Mouse CD11c (223H7)
M100-4 FITC labeled Mouse CD11c (223H7)
D202-3 Mouse CD11b (1C4)
D202-4 FITC labeled Mouse CD11b (1C4)

INTENDED USE:
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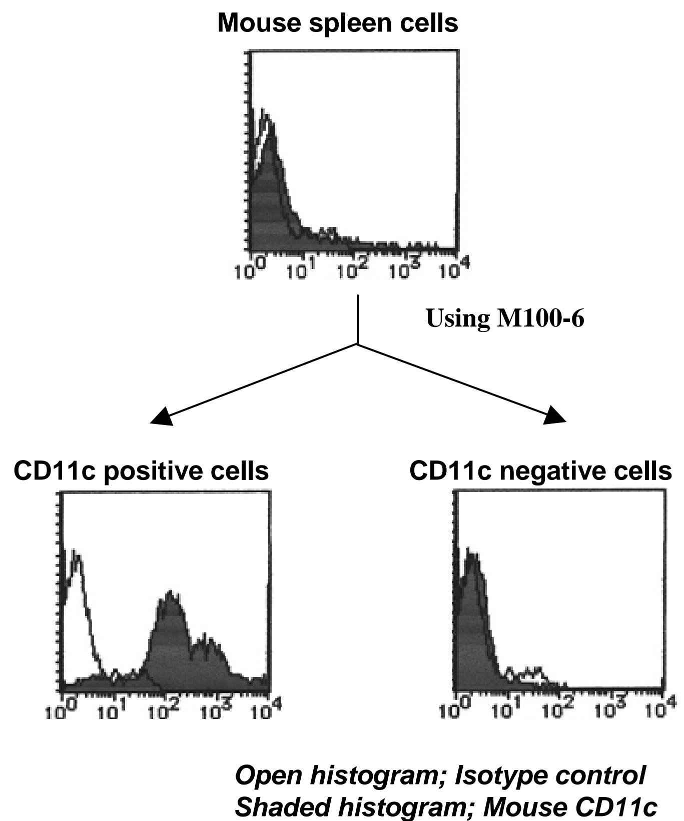
SPECIES CROSS REACTIVITY:

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

REFERENCE:
1) Kruger, T., *et al., J. Am. Soc. Nephrol* **15**, 613-621 (2004)



Open histogram; Isotype control
Shaded histogram; Mouse CD11c

PROTOCOL:
Cell separation using magnetic beads
1) Isolate single cell suspension from mouse spleen by standard preparation method.
2) Wash the cells twice with washing buffer [PBS containing 0.5% BSA and 2 mM EDTA].
3) Resuspend the cells with washing buffer (1x10^8 cells/mL).

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

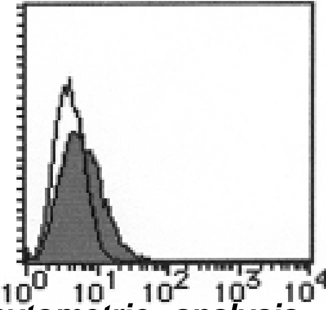
4) Add 20 μL of anti-mouse CD16/32 antibody (MBL; code no. 732121) to the cell suspension for blocking FcR, and incubate for 15 minutes at 4°C.
5) Add 2 μg per $2.5x10^7$ cells of Biotin labeled mouse CD11c (223H7) to the cell suspension, and incubate for 30 minutes at 4°C. Remove supernatant by careful aspiration.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at 4°C. Remove supernatant by careful aspiration.
7) Resuspend cell pellet and label with streptavidin or anti-biotin magnetic beads according to the manufacturer's recommendation.
8) Wash the separated cells with washing buffer.
9) Add 20 μL of 1:40 diluted anti-mouse CD16/32 antibody with washing buffer to the cell suspension, and incubate for 10 minutes at 4°C.
10) Add 20 μL of PE labeled mouse CD11c (10 μg/mL, BD Biosciences). Mix well and incubate for 1 hour at 4°C.
11) 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.
12) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; mouse splenocyte)

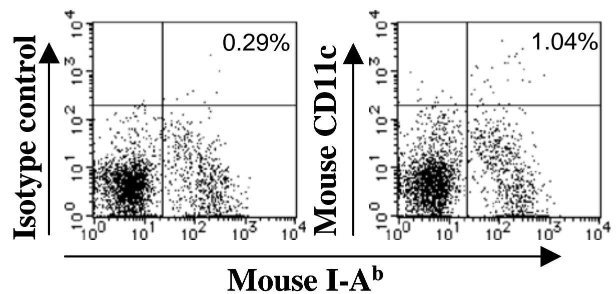
Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all step after 2).

1) Detach the cells from culture dish by cell dissociation buffer.
2) Wash the cells 3 times with washing buffer [PBS containing 0.5% BSA and 2 mM EDTA].
3) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
4) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at 4°C. Remove supernatant by careful aspiration.
5) Add 20 μL of 1:40 diluted anti-mouse CD16/32 antibody (MBL; code no. 732121) with washing buffer to the cell suspension. Mix well, and incubate for 10 minutes at 4°C.
6) Add 1 μg of the Biotin labeled Mouse CD11c monoclonal antibody (223H7). Mix well, and incubate for 30 minutes at 4°C.
7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at 4°C. Remove supernatant by careful aspiration.
8) Add 30 μL of 1:40 diluted PE conjugated streptoavidin (MBL; code no. IM-0557) with the washing buffer. Mix well and incubate for 15 minutes at 4°C.
9) 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.
10) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.



Flow cytometric analysis of Mouse CD11c expression on JAWSII cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of M100-6 to the cells.*



Flow cytometric analysis of Mouse CD11c (right) and isotypic control (left) expression on Mouse splenocyte. *The staining intensity of M100-6 and isotypic control are shown in the vertical axis with Mouse I-A^b staining on the horizontal axis.*