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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

FITC labeled Mouse CD11c

Code No.	Clone	Subclass	Quantity	Concentration
M100-4	223H7	Rat IgG2a	100 µL	500 µg/mL

BACKGROUND: The CD11c (αX 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: 50 µg IgG in 100 µL 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 mouse CD11c antigen on Flow cytometry.

APPLICATIONS:
Flow cytometry; 10 µg/mL (final concentration)
*Please refer to the data sheet (MBL code no. M100-3) for other applications.

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

SPECIES CROSS REACTIVITY:

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

INTENDED USE:
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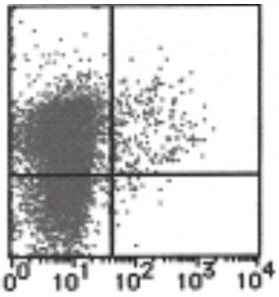
REFERENCES:
1) Fancke, B., *et al.*, *Blood* Oct 2007; 10.1182/blood-2007-05-089292.
2) Kruger, T., *et al., J. Am. Soc. Nephrol.* **15**, 613-21 (2004)

Clone 223H7 is used in reference number 1).



Flow cytometric analysis of Mouse CD11c expression on Mouse splenocytes. *The staining intensity of M100-4 is shown in the horizontal axis with Mouse I-A^b staining on the vertical axis.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:
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 20 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 FITC labeled mouse CD11c monoclonal antibody (223H7) (10 μg/mL) 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 1:50 Biotin labeled anti-mouse I-A^b ($A^a\beta^b$) (BD Biosciences; code no. 06042D) 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 PE labeled streptavidin (MBL; code no. IM-0557) 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) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; mouse splenocyte)