

## Product datasheet for **AM26635BT-N**

### Itgax (alpha) Rat Monoclonal Antibody [Clone ID: 223H7]

#### Product data:

Product Type:	Primary Antibodies
Clone Name:	223H7
Applications:	FC
Recommended Dilution:	Cell separation: 20 µl/2.5x10 <sup>7</sup> cells (ready for use). Flow cytometry: 2 µg/2.5x10 <sup>6</sup> cells. For details see protocol below.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Murine DC from C57BL/6 mice
Specificity:	This antibody reacts with mouse CD11c.
Formulation:	PBS Label: Biotin State: Liquid Ig fraction Stabilizer: 1% BSA Preservative: 0.09% NaN <sub>3</sub>
Concentration:	lot specific
Purification:	Protein G agarose
Conjugation:	Biotin
Storage:	Store at 2-8 °C.
Stability:	Shelf life: one year from despatch.
Gene Name:	integrin alpha X
Database Link:	<a href="#">Entrez Gene 16411 Mouse</a> <a href="#">Q9QXH4</a>



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<b>Background:</b>	The CD11c (integrin $\alpha$ X; ~150 kDa) glycoprotein non-covalently associates with CD18 (integrin $\beta$ 2; ~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.
<b>Synonyms:</b>	ITGAX, Integrin alpha-X, Leu M5

**Note:** This product was originally produced by MBL International.

Protocol: Cell separation using magnetic beads

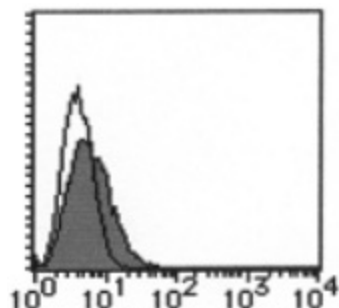
- 1) Isolate single cell suspension from mouse spleen by standard preparation method.
- 2) Wash the cells twice with washing buffer [PBS containing 0.5% BSA and 2 mM EDTA].
- 3) Resuspend the cells with washing buffer (1x10<sup>8</sup> cells/mL).
- 4) Add 20 µL of anti-mouse CD16/32 antibody to the cell suspension for blocking FcR, and incubate for 15 minutes at 4 °C.
- 5) Add 2 µg per 2.5x10<sup>7</sup> 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). 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 steps 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<sup>6</sup> 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 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 streptavidin 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.

## Product images:



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 AM26635BT-N to the cells.