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MBL
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MONOCLONAL ANTIBODY

Anti-Mincle (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D292-3	4A9	Rat IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Macrophage-inducible C-type lectin (Mincle, also called as Clec4e and Clecsf9), a type II transmembrane C-type lectin receptor, is expressed mainly in macrophages. Mincle is selectively associated with the Fc receptor common γ-chain which contains immunoreceptor tyrosine-based activation motif (ITAM) and activates macrophages to produce inflammatory cytokines and chemokines. Mincle expressing cells are activated by spliceosome associated protein 130 (SAP130) released from dead cells. Recently, Mincle is reported to recognize *Mycobacterium tuberculosis* as well as pathogenic fungus *Malassezia* species. Mincle is demonstrated to be an essential receptor for a mycobacterial glycolipid, trehalose-6,6'-dimycolate (TDM). The cytokine/chemokine production of macrophages which *Malassezia* or *M.tuberculosis* induces in Mincle$^{-/-}$ mice is significantly impaired. Mincle is considered to play a crucial role in immune responses to these ligands.

SOURCE: This antibody was purified from hybridoma (clone 4A9) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Wister rat splenocyte immunized with RBL-2H3 cells expressing full length mouse mincle.

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 Mincle on Western blotting, Immunoprecipitation and Flowcytometry.

APPLICATIONS:

Western blotting; 1-5 µg/mL *
*D266-3 is suitable for this application.
Immunoprecipitation; 5-10 µg/200 µL of cell extract from 1 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)
Functional activity; 1-10 µg/mL*
*It is reported that this antibody has functional activity in the reference number 2) and 5).

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Not tested	LPS stimulated mouse macrophage	Not tested
Reactivity on IP and FCM		+	

INTENDED USE:
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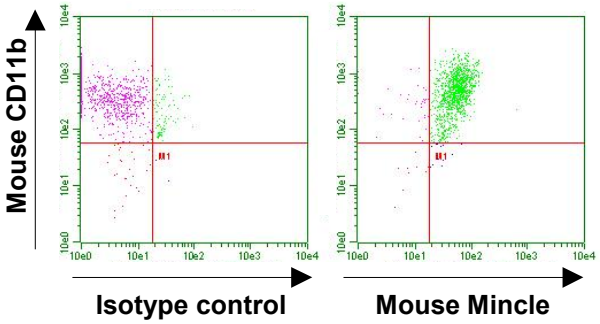
REFERENCES:

1) Toyonaga, K., *et al., PLoS One* **9**, e88747 (2014) [FCM]
2) Miyake, Y., *et al. Immunity* **38**, 1050-1062 (2013) [FCM, Function]
3) Yamasaki, S., *et al., PNAS.* **106**, 1897 -1902 (2009)
4) Ishikawa, E., *et al., J. Exp. Med.* **206**, 2879-2888 (2009)
5) Yamasaki, S., *et al., Nat. Immunol.* **9**, 1179-1188 (2008) [FCM, Function]

Clone 4A9 is used in the reference number 1)-2) and 5).



Flow cytometric analysis of isotypic control (left) and mouse Mincle expression (right) on LPS stimulated mouse macropages.
The staining intensitiy of mouse CD11b is shown in the vertical axis with D292-3 staining on the horizontal axis.

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

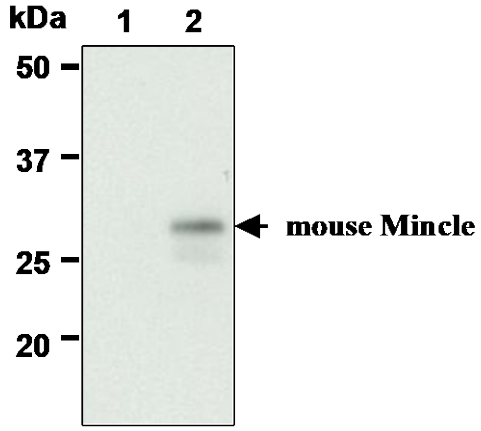
PROTOCOL:

Flow cytometric analysis for floating cells

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

1) Wash the cells 1 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 100 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 30 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
5) 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.
6) Add FITC conjugated anti-rat IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
7) 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.
8) Add PE conjugated mouse CD11b/Mac-1 diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
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.

(Positive control for Flow cytometry; LPS stimulated mouse macrophage)



Immunoprecipitation from LPS stimulated mouse macrophage with isotypic control (1) or D292-3 (2).
After immunoprecipitated with the antibody, immunocomplexes were resolved on SDS-PAGE and immunoblotted with D292-3.

Immunoprecipitation

1) Wash cells (approximately 5 x 10^6 cells) 3 times with PBS and resuspend them in 1 mL of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing protease inhibitors at appropriate concentrations. Incubate it at 4°C with rotating for 30 minutes; thereafter, briefly sonicate the mixture (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 fresh tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 60-120 minutes at 4°C. Add 20 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) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; LPS stimulated mouse macrophage)

SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 20 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out 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 manufacturer'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 for 1 hour at room temperature with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with 1:10,000 Anti-IgG (Rat) pAb-HRP (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 off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Western blotting; Transfectant)

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