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Not for use in diagnostic procedures.

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Anti-Mincle (Mouse) mAb

CODE No.	D266-3
CLONALITY	Monoclonal
CLONE	1B6
ISOTYPE	Rat IgG1 κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	RBL-2H3 cells expressing full-length mouse mincle
REACTIVITY	This clone reacts with mouse Mincle (Clec4e) and crossreacts weakly with MCL (Clec4d).
FORMULATION	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.

APPLICATIONS-CONFIRMED

Western blotting	1 µg/mL for chemiluminescence detection system
Immunoprecipitation	2 µg/2 x 10^6 cells/sample
Flow cytometry	2 µg/mL

APPLICATION-REPORTED

Functional activity	1-10 µg/mL for blocking, Reference 1) and 2)

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Guinea Pig
Cell	Not tested	LPS-stimulated Balb/c mouse peritoneal macrophage	Not tested	Not tested
Reactivity		+		

Entrez Gene ID 56619 (Mouse)

REFERENCES

1) Yamasaki, S., *et al*., *Nat. Immunol*. **9**, 1179-1188 (2008) [WB, FCM, Function]
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)

RELATED PRODUCTS

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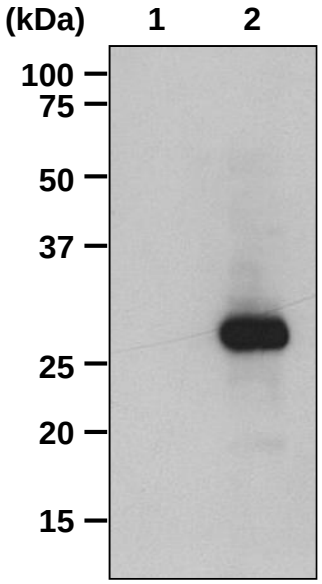
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 2 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. 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 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with the 1:10,000 of anti-IgG (Rat) pAb-HRP (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; LPS-stimulated Balb/c mouse peritoneal macrophage)



Western blot analysis of mouse Mincle expressed in Balb/c mouse peritoneal macrophages

Lane 1: Non-stimulation
Lane 2: LPS-stimulated

Immunoblotted with Anti-Mincle (Mouse) mAb (D266-3)

Immunoprecipitation

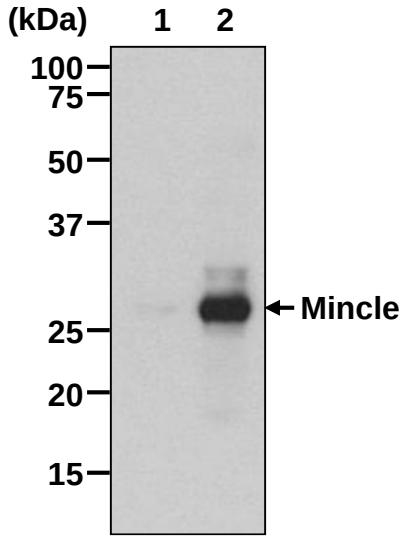
[Preparation of antibody-bound protein G beads]

a) Mix 20 µL of 50% protein G agarose beads slurry resuspended in 500 µL of IP buffer (10 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at room temperature.
b) Wash the beads 3 times with 1 mL of IP Buffer.
c) Wash the beads 3 times with 1 mL of Fixation Buffer (0.1 M $Na_2B_4O_7$)
d) Add 200 µL of 20 mM Dimethyl pimelimidate (DMP) in Fixation buffer. Incubate with gentle agitation for 30 min. at room temperature.
e) Centrifuge the tube at 300 x g for 2 min. at 4°C. Carefully discard the sup and add 200 µL of 200 mM Tris-HCl, pH 8.0. Incubate at 4°C until just before use.
f) Wash the beads 3 times with 1 mL of 200 mM Tris-HCl, pH 8.0.
g) Wash the beads 1 time with 1 mL of IP buffer.

[Protocol]

1) Wash 4 x 10^6 cells 2 times with PBS and resuspend them with 1 mL of Extraction buffer (50 mM Tris-HCl pH 7.4, 150mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 15 sec.).
2) Incubate the tube for 10 min. on ice.
3) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
4) Add 500 µL of the supernatant to the tube containing antibody conjugated beads (Step g)).
5) Incubate with gentle agitation for 1 hr. at room temperature.
6) Wash the beads 6 times with 1 mL of Extraction buffer.
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge. Use 10 µL/lane for the SDS-PAGE analysis. (See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; LPS-stimulated Balb/c mouse peritoneal macrophage)



Immunoprecipitation of mouse Mincle from LPS-stimulated Balb/c mouse peritoneal macrophages

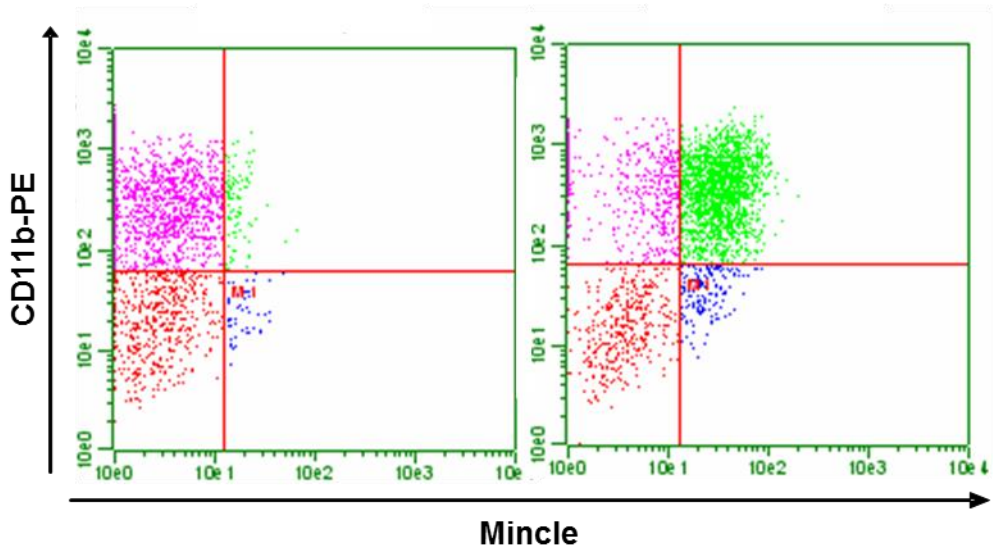
Lane 1: Isotype control (M080-3)
Lane 2: Anti-Mincle (Mouse) mAb (D266-3)

Immunoblotted with D266-3

Flow cytometric analysis

1) Wash the cells (2 x 10^5 cells/sample) 2 times with 1 mL of washing buffer (PBS containing 2% fetal calf serum (FCS)).
2) Add 20 µL of 1 mg/mL human IgG in normal goat serum to the cell pellet after tapping. Mix well and incubate for 5 min. at room temperature.
3) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
4) Wash the cells 1 time with 1 mL of the washing buffer.
5) Add 40 µL of 1:100 anti-IgG (Rat) pAb-FITC (MBL; code no. IM-0827) diluted with the washing buffer. Mix well and incubate for 15 min. at room temperature.
6) Wash the cells 1 time with 1 mL of the washing buffer.
7) Add 40 µL of 1:100 CD11b (Mac-1)-PE (Beckman Coulter; code no. 732048) diluted with the washing buffer. Mix well and incubate for 15 min. at room temperature.
8) Wash the cells 1 time with 1 mL of the washing buffer.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LPS-stimulated Balb/c mouse peritoneal macrophage)



Flow cytometric detection of mouse Mincle in LPS-stimulated Balb/c mouse peritoneal macrophages

Left: Isotype control (M080-3)
Right: Anti-Mincle (Mouse) mAb (D266-3)