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Not for use in diagnostic procedures.

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Anti-Mincle (Human) mAb

(Functional Grade)

CODE No. D360-3

CLONALITY Monoclonal
CLONE 1H2
ISOTYPE Rat IgG2b κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Human Mincle expressed 293T transfectants
FORMULATION 1 mg/mL in PBS. Azide free, 0.22 µm sterile-filtered
Endotoxin level is <0.5 EU/mg antibody, as determined by the LAL assay
STORAGE This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Flow cytometry	1-5 µg/mL for chemiluminescence detection system
Functional activity	1 µg/mL for inhibition assay

SPECIES CROSS REACTIVITY on FCM

Species	Human	Mouse	Rat	Hamster
Cell	Transfectant	Not tested	Not tested	Not tested
Reactivity	+			

Entrez Gene ID 26253 (Human)

REFERENCE
1) Hattori, Y., *et al., J. Biol. Chem.* **289**, 15405-15412 (2014) [Function]
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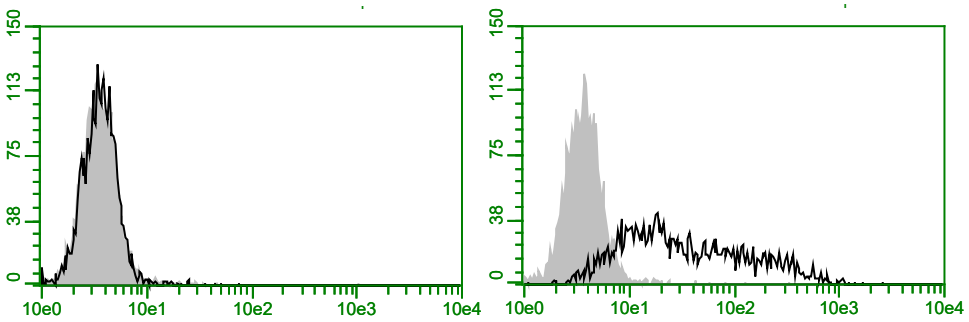
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Flow cytometric analysis

1) Wash the cells (5 x 10^5 cells/sample) 3 times with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
2) Add 10 μL of Clear Back (MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 10 min. at room temperature.
3) Add 40 μL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in washing buffer. Mix well and incubate for 20 min. at room temperature.
4) Wash the cells 1 time with 1 mL of washing buffer.
5) Add 40 μL of FITC-conjugated anti-rat IgG antibody 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) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Human Mincle and Human FcRγ co-transfected HEK293T cells)



Flow cytometric detection of human Mincle in HEK293T transfectant

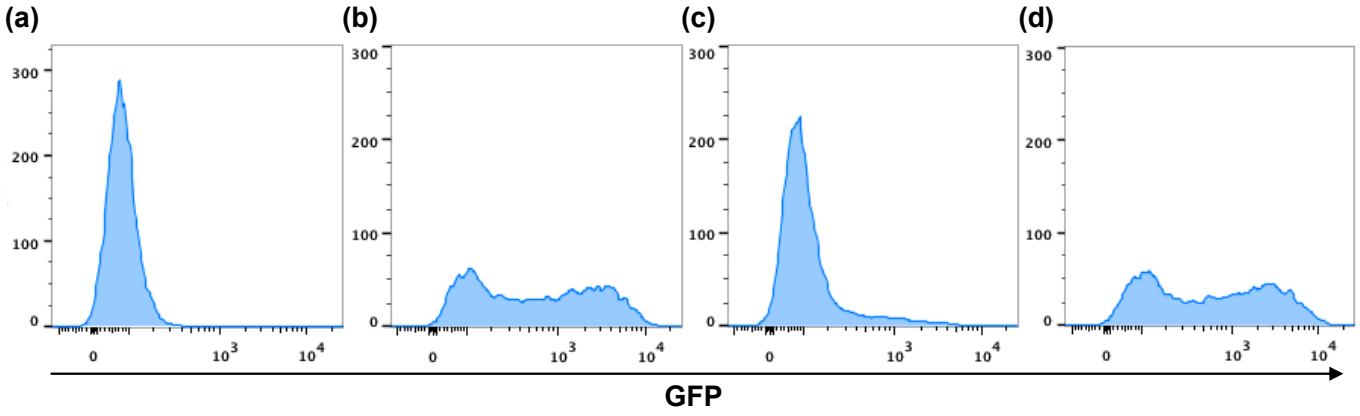
Left: HEK293T cells
Right: Human Mincle and Human FcRγ co-transfected HEK293T cells

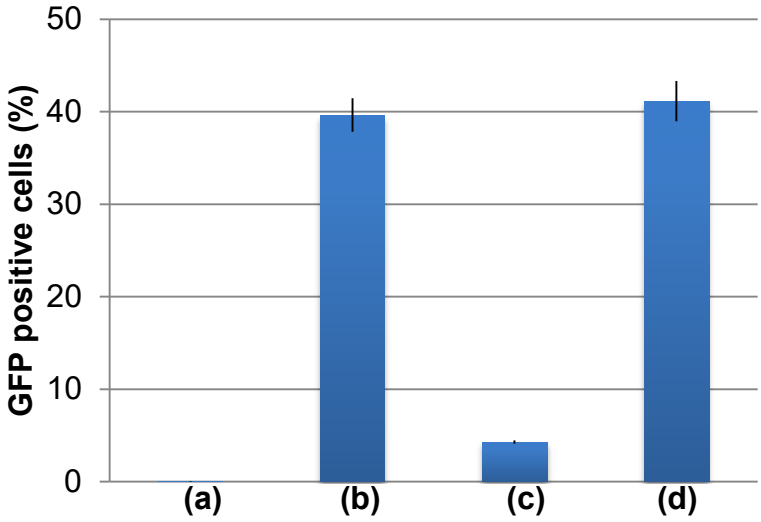
Open: Anti-Mincle (Human) mAb (D360-3), 1 μg/mL
Closed: Isotype control (M090-3)

Inhibition assay

1) Dissolve trehalose dimycolate (TDM) to 1 mg/mL in chloroform/methanol (2:1, v/v) and dilute 1:1,000 with isopropanol.
2) Add 30 µL of TDM solvent (Step 1) to the 96-well microtiter plates.
3) Evaporate the soluvent in safety cabinet until drying.
4) Incubate 5 x 10^4 cells of 2B4-NFAT-GFP reporter cells expressing wild-type human Mincle in the pre-coated or non-coated plates.
5) Add the primary antibody as suggested in the **APPLICATIONS** to the wells and mix well.
6) Incubate at 37°C, 5% CO_2 for 18 hr.
7) Harvest the cells and add propidium iodide to gate out dead cells.
8) The cell samples were then analyzed for GFP-positive cells by Flow cytometry.

(Positive control for Flow cytometry; TDM-stimulated 2B4-NFAT-GFP reporter cells expressing wild-type human Mincle)





Inhibition assay of human Mincle

(a) Unstimulated cells
(b) TDM-stimulated cells
(c) TDM-stimulated cells + Anti-Mincle (Human) mAb (D360-3)
(d) TDM-stimulated cells + Rat IgG2b (isotype control) (M090-3)

Data were kindly provided by Dr. Masahiko Sugita. (Institute for Virus Research, Kyoto University)