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Not for use in diagnostic procedures.

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Anti-FcμR (TOSO/FAIM3) (Mouse) mAb

CODE No. D303-3

CLONALITY Monoclonal
CLONE #4B5
ISOTYPE Rat IgG1 κ
QUANTITY 100 μL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN Mouse FcμR extracellular domain-Fc fusion protein
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

Immunocytochemistry	10 μg/mL
Flow cytometry	10 μg/mL

SPECIES CROSS REACTIVITY on FCM

Species	Human	Mouse	Rat	Hamster
Cells	Transfectant	Transfectant Splenocytes* Bone marrow cells*	Not tested	Not tested
Reactivity	–	+		

*Reactivity of this clone to mouse splenocytes and bone marrow cells on FCM is described in the reference number 1).

Entrez Gene ID 69169 (Mouse)

REFERENCES

1) Shima, H., *et al*., *Int. Immunol*. **22**, 149-156 (2010) [FCM]
2) Kubagawa, H., *et al*., *J. Exp. Med*. **206**, 2779-2793 (2009)
3) Pallasch, C. P., *et al*., *Blood* **112**, 4213-4219 (2008)
4) Proto-Siqueira, R., *et al*., *Blood* **112**, 394-397 (2008)
5) Hitoshi, Y., *et al*., *Immunity* **8**, 461-471 (1998)

RELATED PRODUCTS

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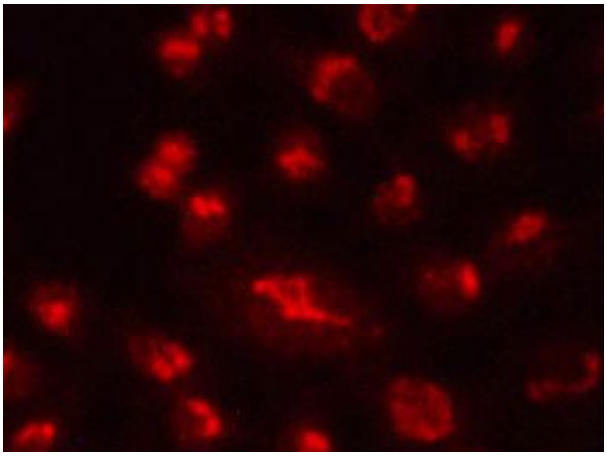
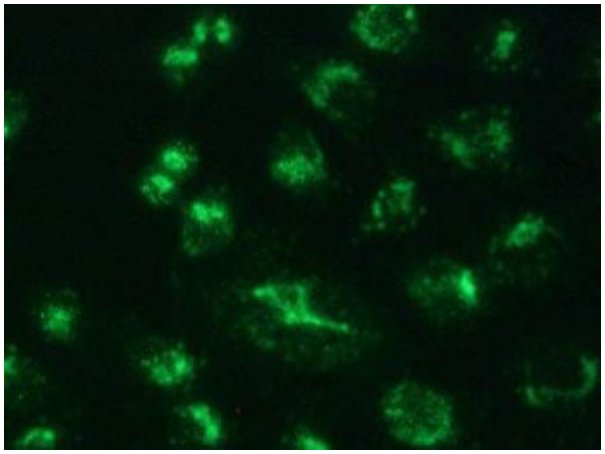
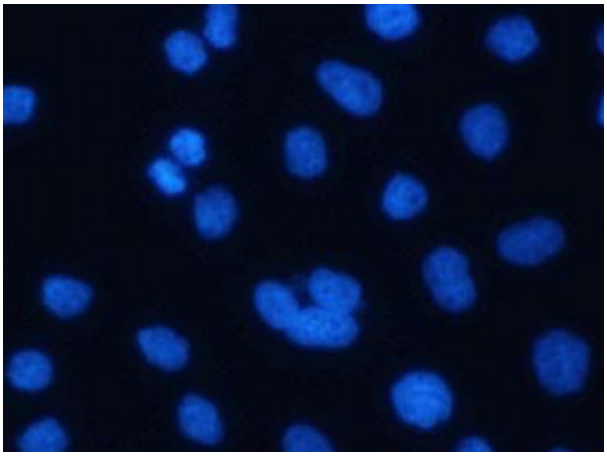
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Immunocytochemistry

1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
4) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 min. Take care not to touch the cells. Repeat another wash once more.
5) Immerse the slide in 0.2% Triton X-100/PBS for 20 min. at room temperature.
6) Wash the slide in a plenty of PBS as in the step 4).
7) Add 200 µL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the slide in a plenty of PBS as in the step 4).
9) Add 200 µL of 1:500 Alexa Fluor® 594 conjugated anti-rat IgG (Thermo Fisher Scientific, code no. A-11007) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
10) Wash the slide in a plenty of PBS as in the step 4).
11) Add 200 µL of 0.5 µg/mL Anti-HA-tag mAb-Alexa Fluor® 488 (MBL, code no. M180-A48) diluted with PBS onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
12) Wash the slide in a plenty of PBS as in the step 4).
13) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
14) Counter stain with DAPI for 5 min. at room temperature.
15) Wash the slide in a plenty of PBS as in the step 4).
16) Promptly add mounting medium onto the slide, then put a cover slip on it.





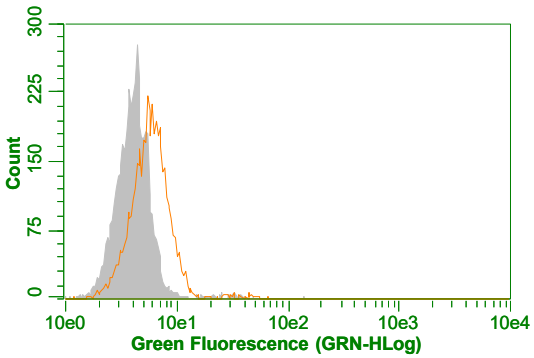


Immunocytochemical detection of HA-tagged mouse FcμR/TOSO/FAIM3 in HeLa

Red: D303-3
Green: Anti-HA-tag mAb-Alexa Fluor® 488
(MBL, code no.M180-A48)
Blue: DAPI

Flow cytometric analysis for adherent cells

1) Detach the cells from culture dish.
2) Wash the cells once with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
3) Resuspend the cells with washing buffer (3 x 10^6 cells/mL).
4) Add 100 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 min. at room temperature (20~25°C). Remove supernatant by careful aspiration.
5) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 10 min. at room temperature.
6) 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 20 min. at 4°C.
7) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration. Repeat another wash once more.
8) Add 30 µL of 1:100 FITC conjugated anti-rat IgG diluted with the washing buffer. Mix well and incubate for 20 min. at room temperature.
9) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. 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.



Flow cytometric detection of HA-tagged mouse Fcµ R/TOSO/FAIM3 in HeLa

Open: D303-3
Closed: Isotype control (MBL, code no. M080-3)