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Anti-FcεR1γ (FcRγ) (Mouse) mAb

CODE No. M191-3

CLONALITY Monoclonal
CLONE 1D6
ISOTYPE Mouse IgG1 κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
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	0.2 µg/mL for chemiluminescence detection system
Immunoprecipitation	2 µg/200 µL of cell extract from 2 x 10^6 cells
Immunocytochemistry	1 µg/mL
Flow cytometry	1 µg/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	293T, HUVEC, HL-60, Raji	Transfectant, Mouse peritoneal macrophage, WEHI-3B	Not tested	Not Tested
Reactivity	–	+		

Entrez Gene ID 14127 (Mouse)

REFERENCES

1) Yamasaki, S., *et al.*, *Nat. Immunol.* **9**, 1179-1188 (2008)
2) Cao, L., *et al., J.Immunol.* **179**, 5864-5876 (2007)
3) Sato, K., *et al.*, *J. Biol. Chem.* **281**, 38854-38866 (2006)
4) Ra, C., *et al.*, *J. Biol. Chem.* **264**, 15323-15327 (1989)

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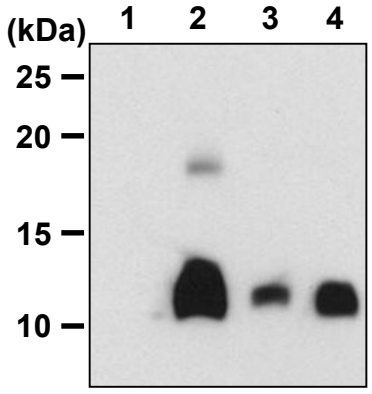
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

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 seconds).
2) Boil the samples for 3 minutes 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 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 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 minutes x 3 times).
8) Incubate the membrane with the 1:10,000 anti-IgG (Mouse)-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
9) Wash the membrane with PBS-T (5 minutes x 3 times).
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Trasnfectant, mouse peritoneal macrophage and WEHI-3B)

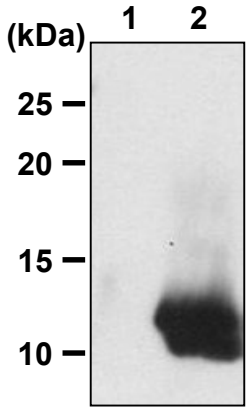


Western blot analysis of FcεR1γ (Mouse)
Lane 1: Parental cell (293T)
Lane 2: Mouse FcεR1γ/293T
Lane 3: Mouse peritoneal macrophage
Lane 4: WEHI-3B
Immunoblotted with M191-3

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors, then sonicate briefly (up to 20 seconds).
2) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C and transfer the supernatant to another tube.
3) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 200 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 gently agitation for 1 hour at room temperature.
4) Wash the beads 3 times with 1 mL of IP buffer.
5) Add 200 µL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hour at room temperature.
6) Wash the beads 5 times with 1 mL of Lysis buffer.
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 2 minutes and centrifuge.
8) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
9) 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.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
12) Incubate the membrane with 1 µg/mL anti-FcεR1γ (Mouse) pAb (MBL; code no. PM068) diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS-T (5 minutes x 3 times).
14) Incubate the membrane with the 1:10,000 anti-IgG (Rabbit)-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
15) Wash the membrane with PBS-T (5 minutes x 3 times).
16) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 minute.
17) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
18) Expose to an X-ray film in a dark room for 1 minute. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Mouse peritoneal macrophage)

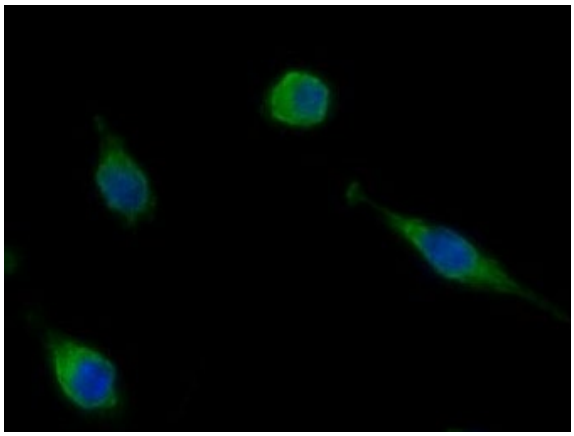


Immunoprecipitation of FcεR1γ (Mouse)
from mouse peritoneal macrophage
Lane 1: IP with isotype control (M075-3)
Lane 2: IP with M191-3
Immunoblotted with Anti-FcεR1γ (Mouse) mAb (PM068)

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 minutes at room temperature (20~25ºC).
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 minutes. Take care not to touch the cells. Repeat another wash once more.
5) Immerse the slide in 0.2% Triton X-100/PBS for 10 minutes at room temperature.
6) Wash the slide in a plenty of PBS as in the step 4).
7) Add Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell and incubate for 5 minutes at room temperature.
8) 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 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
9) Wash the slide in a plenty of PBS as in the step 4).
10) Add 100 µL of 1:500 anti-IgG (Mouse)-Alexa Fluor®488 (Invitrogen; code no. A11001) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
11) Wash the slide in a plenty of PBS as in the step 4).
12) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
13) Counter stain with DAPI for 5 minutes at room temperature.
14) Wash the slide in a plenty of PBS as in the step 4).
15) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; RAW264)

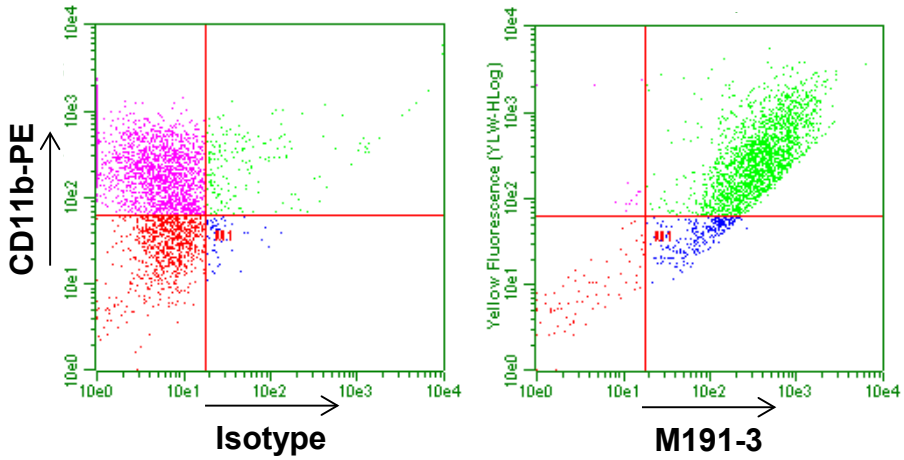


Immunocytochemical detection of FcεR1γ (Mouse) in RAW264
Green: M191-3
Blue: DAPI

Flow cytometric analysis

1) Wash the cells (3 x 10^5 cells/sample) 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
2) Add 4% paraformaldehyde (PFA)/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
3) Wash the cells 2 times with 1 mL of washing buffer.
4) Add 0.2% Triton X-100 in PBS to the cell pellet after tapping. Mix well, then permeabilize the cells for 5 min. at room temperature.
5) Wash the cells 1 time with 1 mL of washing buffer.
6) 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 minutes at room temperature.
7) 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 minutes at 4°C.
8) 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. Repeat another wash once more.
9) Add 30 µL of 1:100 anti-IgG (Mouse)-Alexa Fluor®488 (Invitrogen; code no. A11001) diluted with the washing buffer. Mix well and incubate for 20 minutes at room temperature.
10) 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.
11) Add 30 µL of 1:200 anti-CD11b (Mouse)-PE (Beckman Coulter; code no. 732048) diluted in the washing buffer. Mix well and incubate for 20 minutes at room temperature.
12) 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.
13) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse peritoneal macrophage)



Flow cytometric detection of FcεR1γ (Mouse) in mouse peritoneal macrophage

Left: isotype control (M075-3)
Right: M191-3