

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

<u>Western blotting</u>	0.2 μg/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	2 μg/200 μL of cell extract from 2 x 10 ⁶ cells
<u>Immunocytochemistry</u>	1 μg/mL
<u>Flow cytometry</u>	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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RELATED PRODUCTS

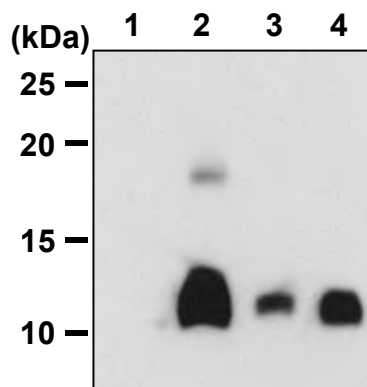
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 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 μ L 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/cm² 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; Transfectant, mouse peritoneal macrophage and WEHI-3B)



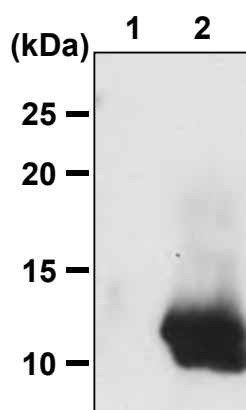
Western blot analysis of FcεR1γ (Mouse)

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 1×10^7 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 μ L of 50% protein A agarose beads slurry resuspended in 200 μ L 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 hour at room temperature.
- 4) Wash the beads 3 times with 1 mL of IP buffer.
- 5) Add 200 μ L 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 μ L of Laemmli's sample buffer, boil for 2 minutes and centrifuge.
- 8) Load 10 μ L 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/cm² 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 μ g/mL anti-Fc ϵ R1 γ (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)



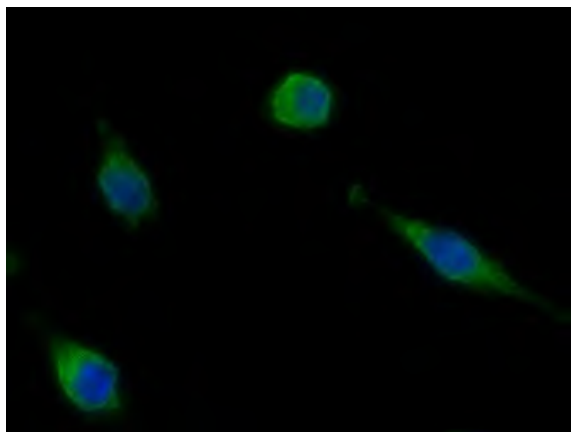
Immunoprecipitation of Fc ϵ R1 γ (Mouse) from mouse peritoneal macrophage

Lane 1: IP with isotype control (M075-3)
Lane 2: IP with M191-3
Immunoblotted with Anti-Fc ϵ R1 γ (Mouse) mAb (PM068)

Immunocytochemistry

- 1) Spread the cells on a glass slide, then incubate in a CO₂ 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 (20~25°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 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

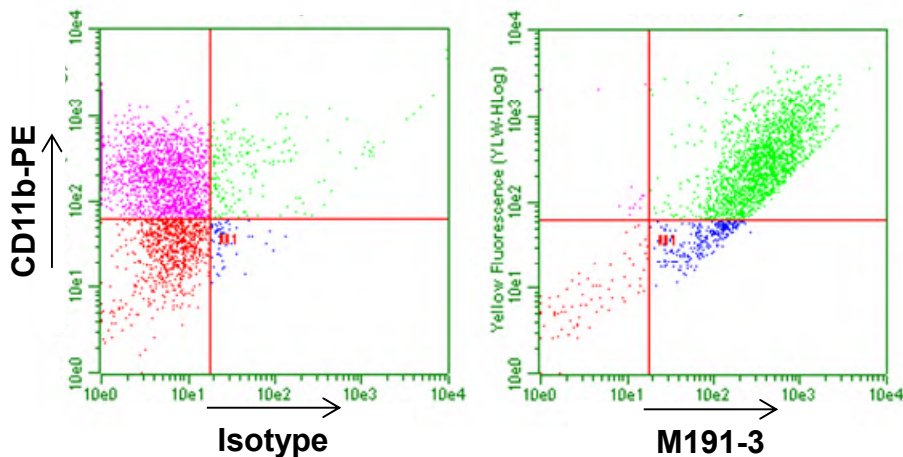
Green: M191-3

Blue: DAPI

Flow cytometric analysis

- 1) Wash the cells (3×10^5 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 μ L 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 μ L 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 μ L 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 μ L 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 μ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse peritoneal macrophage)



Flow cytometric detection of Fc ϵ R1 γ (Mouse) in mouse peritoneal macrophage

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