

# Anti-FcεR1γ (FcRγ) (Mouse) mAb -Alexa Fluor<sup>®</sup> 647

**CODE No.** M191-A64

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

**SOURCE** Purified IgG from hybridoma supernatant  
**FORMULATION** PBS containing 1% BSA and 0.09% NaN<sub>3</sub>

\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

**STORAGE** This antibody solution is stable for one year from the date of purchase when stored at 4°C.

## APPLICATIONS-CONFIRMED

Immunocytochemistry 1 μg/mL  
Flow cytometry 1 μg/mL

## SPECIES CROSS REACTIVITY

Species	Human	Mouse	Rat	Hamster
Cell	Not tested	RAW264, Mouse peritoneal macrophage	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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## LABEL LICENSES:

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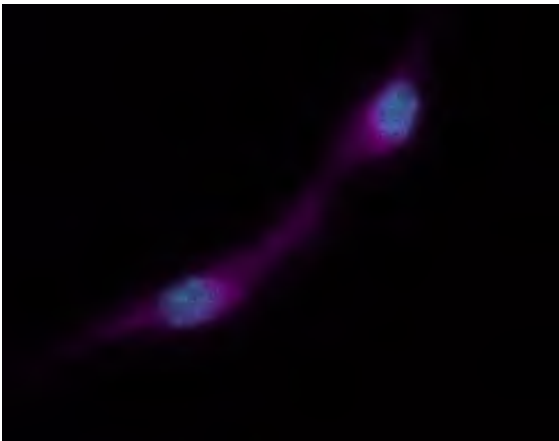
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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

### **Immunocytochemistry**

- 1) Spread the cells on a glass slide, then incubate in a CO<sub>2</sub> 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 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 10 min. at room temperature.
- 6) Wash the slide twice in PBS for 5 min. each.
- 7) Add Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell and incubate for 5 min. at room temperature.
- 8) Add 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.)
- 9) Wash the slide twice in PBS for 5 min. each.
- 10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 11) Counter stain with DAPI for 5 min. at room temperature.
- 12) Wash the slide twice in PBS for 5 min. each.
- 13) 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***

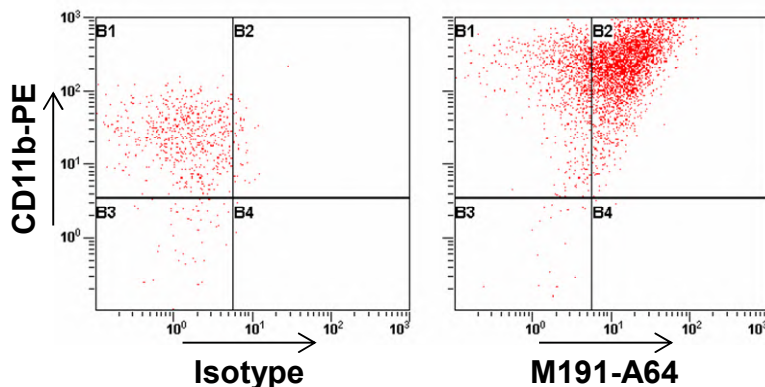
Magenta: M191-A64

Cyan: DAPI

**Flow cytometric analysis**

- 1) Wash the cells ( $5 \times 10^5$  cells/sample) 3 times 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 twice 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 10 min. at room temperature.
- 5) Wash the cells twice with 1 mL of washing buffer.
- 6) Add 20  $\mu$ 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 5 min. at room temperature.
- 7) Add 40  $\mu$ L 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.
- 8) Wash the cells 1 time with 1 mL of washing buffer.
- 9) Add 40  $\mu$ L of 1:200 anti-CD11b (Mouse)-PE (Beckman Coulter; code no. 732048) diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
- 10) Wash the cells 1 time with 1 mL of washing buffer.
- 11) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Mouse peritoneal macrophage)



***Flow cytometric detection of  $Fc\epsilon R1\gamma$  (Mouse) in mouse peritoneal macrophage***

Left: isotype control (M075-A64)

Right: M191-A64