

**For Research Use Only.**  
**Not for use in diagnostic procedures.**



## Syrian Hamster IgG (isotype control)

<b>CODE No.</b>	M189-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	ttko
<b>ISOTYPE</b>	Syrian Hamster IgG
<b>QUANTITY</b>	100 µL, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant using protein A agarose.
<b>REACTIVITY</b>	No specific reaction was detected on immunoprecipitation and flow cytometry.
<b>FORMURATION</b>	1 mg/mL in PBS containing 50% glycerol. No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

### APPLICATIONS-CONFIRMED

#### Immunoprecipitation

#### Flow cytometry

This antibody can be used as a negative isotypic control.  
The concentration will depend on the conditions.

For more information, please visit our web site <http://ruo.mbl.co.jp/>



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## RELATED PRODUCTS

### Functional grade antibodies

M075-3M2 Mouse IgG1 (isotype control) FG (2E12)  
M076-3M2 Mouse IgG2a (isotype control) FG (6H3)  
M077-3M2 Mouse IgG2b (isotype control) FG (3D12)  
M078-3M2 Mouse IgG3 (isotype control) FG (6A3)  
M080-3M2 Rat IgG1 (isotype control) FG (1H5)  
M081-3M2 Rat IgG2a (isotype control) FG (2H3)  
M090-3M2 Rat IgG2b (isotype control) FG (3G8)  
M082-3M2 Rat IgG2c (isotype control) FG (6E12)

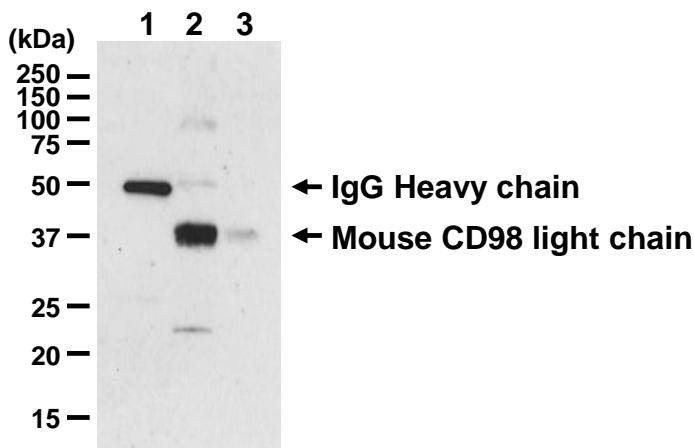
M090-5 Rat IgG2b (isotype control)-PE (3G8)  
M090-A48 Rat IgG2b (isotype control)  
-Alexa Fluor<sup>®</sup> 488 (3G8)  
M082-3 Rat IgG2c (isotype control) (6E12)  
M082-4 Rat IgG2c (isotype control)-FITC (6E12)  
PM035 Normal Rabbit IgG (polyclonal)  
PM035-8 Normal Rabbit IgG-Agarose (polyclonal)  
PM067 Normal Guinea Pig IgG (polyclonal)

### Purified antibodies

M199-3 Armenian Hamster IgG (isotype control)  
M189-3 Syrian Hamster IgG (isotype control)  
M075-3 Mouse IgG1 (isotype control) (2E12)  
M075-4 Mouse IgG1 (isotype control)-FITC (2E12)  
M075-5 Mouse IgG1 (isotype control)-PE (2E12)  
M075-8 Mouse IgG1 (isotype control)-Agarose (2E12)  
M075-9 Mouse IgG1 (isotype control)-Magnetic beads  
(2E12)  
M075-A48 Mouse IgG1 (isotype control)  
-Alexa Fluor<sup>®</sup> 488 (2E12)  
M075-A64 Mouse IgG1 (isotype control)  
-Alexa Fluor<sup>®</sup> 647 (2E12)  
M076-3 Mouse IgG2a (isotype control) (6H3)  
M076-4 Mouse IgG2a (isotype control)-FITC (6H3)  
M076-5 Mouse IgG2a (isotype control)-PE (6H3)  
M076-9 Mouse IgG2a (isotype control)  
-Magnetic beads (6H3)  
M076-A48 Mouse IgG2a (isotype control)  
-Alexa Fluor<sup>®</sup> 488 (6H3)  
M076-A64 Mouse IgG2a (isotype control)  
-Alexa Fluor<sup>®</sup> 647 (6H3)  
M077-3 Mouse IgG2b (isotype control) (3D12)  
M077-4 Mouse IgG2b (isotype control)-FITC (3D12)  
M077-5 Mouse IgG2b isotype control-PE (3D12)  
M077-9 Mouse IgG2b (isotype control)  
-Magnetic beads (3D12)  
M077-A48 Mouse IgG2b (isotype control)  
-Alexa Fluor<sup>®</sup> 488 (3D12)  
M077-A64 Mouse IgG2b (isotype control)  
-Alexa Fluor<sup>®</sup> 647 (3D12)  
M078-3 Mouse IgG3 (isotype control) (6A3)  
M078-4 Mouse IgG3 (isotype control)-FITC (6A3)  
M079-3 Mouse IgM (isotype control) (7E10)  
M194-3 Human IgG1 isotype control chimeric mAb  
M195-3 Human IgG2 isotype control chimeric mAb  
M080-3 Rat IgG1 (isotype control) (1H5)  
M080-4 Rat IgG1 (isotype control)-FITC (1H5)  
M080-5 Rat IgG1 (isotype control)-PE (1H5)  
M080-A48 Rat IgG1 (isotype control)-Alexa Fluor<sup>®</sup> 488 (1H5)  
M081-3 Rat IgG2a (isotype control) (2H3)  
M081-4 Rat IgG2a (isotype control)-FITC (2H3)  
M081-5 Rat IgG2a (isotype control)-PE (2H3)  
M081-8 Rat IgG2a (isotype control)-Agarose (2H3)  
M081-9 Rat IgG2a (isotype control)-Magnetic beads (2H3)  
M081-A48 Rat IgG2a (isotype control)  
-Alexa Fluor<sup>®</sup> 488 (2H3)  
M090-3 Rat IgG2b (isotype control) (3G8)  
M090-4 Rat IgG2b (isotype control)-FITC (3G8)

### **Immunoprecipitation**

- 1) Wash  $1 \times 10^7$  cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Incubate it on ice for 15 min., thereafter, sonicate briefly (up to 15 sec.).
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Add the isotype control antibody at the equal amount of the antibody for immunoprecipitation to the supernatant. Vortex briefly and incubate with gentle agitation for 60-120 min. at 4°C.
- 4) Mix 20  $\mu$ L of 50% protein A agarose beads slurry resuspended in 400  $\mu$ L of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody. Incubate with gentle agitation for 1 hr. at room temperature.
- 5) Wash the beads 3 times with 1 mL of IP buffer.
- 6) Add 300  $\mu$ L of cell lysate (prepared sample of step 2)), then incubate with gentle agitation for 1 hr. at room temperature.
- 7) Wash the beads 6 times with 1 mL of Lysis buffer (centrifuge the tube at 2,500 x g for 10 sec.).
- 8) Resuspend the beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 3 min. and centrifuge for 5 min.
- 9) Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
- 10) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hr. 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.
- 11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 12) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 13) Wash the membrane with PBS (5 min. x 6 times).
- 14) Incubate the membrane with 1:1,000 HRP-conjugated anti-Syrian Hamster IgG (Abcam; code no. Ab7146-100) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 15) Wash the membrane with PBS (5 min. x 6 times).
- 16) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
- 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 3 min. Develop the film as usual. The condition for exposure and development may vary.

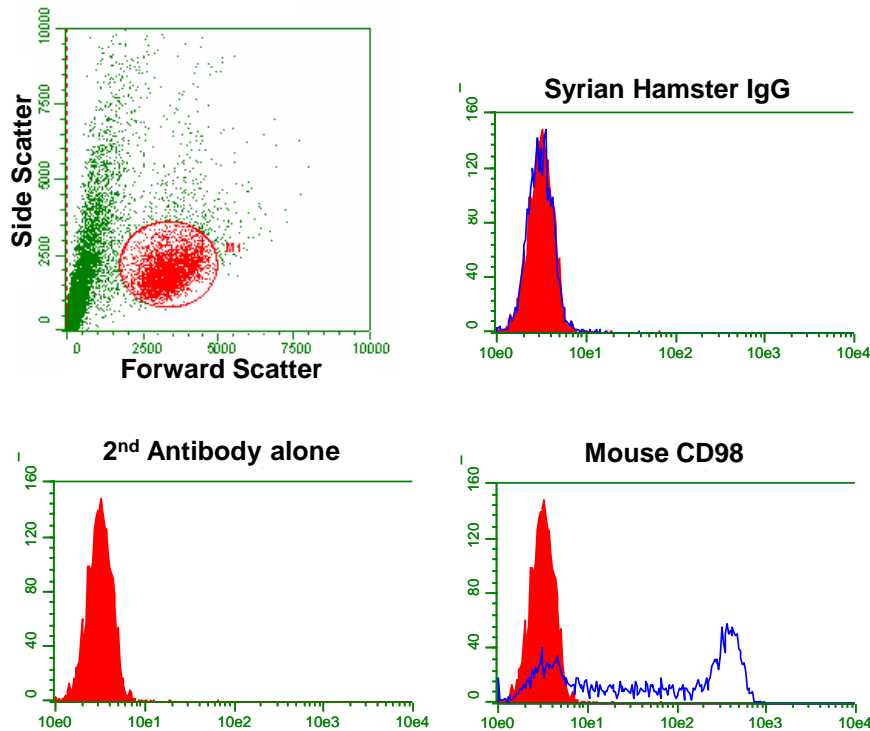


### ***Immunoprecipitation of mouse CD98 from P3U1 cells***

- Lane 1: Syrian Hamster IgG (M189-3)
  - Lane 2: Anti-mouse CD98 mAb (D135-3)
  - Lane 3: Input (cell lysate)
- Immunoblotted with Anti-mouse CD98 mAb (D135-3)

### **Flow cytometric analysis for cells**

- 1) Wash the cells 3 times with 1 mL of the washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Resuspend the cells with the washing buffer ( $5 \times 10^6$  cells/mL).
- 3) Add 100  $\mu$ L 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.
- 4) 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.
- 5) Add the isotype control antibody at the concentrations comparable to those of the specific antibody of interest. The antibody is diluted with the washing buffer. Mix well and incubate for 20 min. at room temperature.
- 6) 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.
- 7) Add 40  $\mu$ L of 1:100 FITC conjugated anti-Hamster IgG (BD Pharmingen; code no. 554011) diluted with the washing buffer. Mix well and incubate for 30 min. at room temperature.
- 8) 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.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.



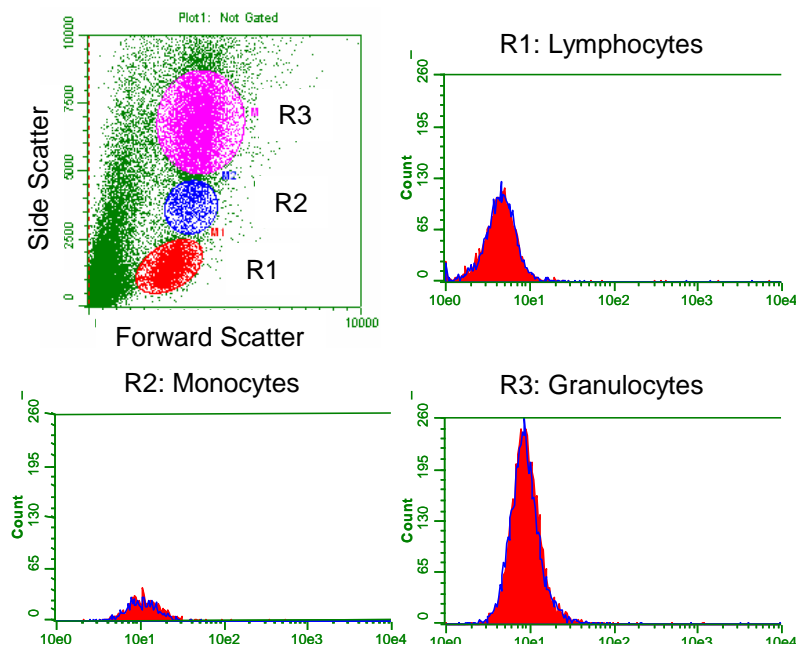
### ***Flow cytometric analysis of mouse CD98 expression on P3U1 cells***

Open: Syrian Hamster IgG (M189-3) or mouse CD98 (D137-3)

Closed: Secondary antibody alone

### Flow cytometric analysis for whole blood cells

- 1) Add the isotype control antibody at the concentrations comparable to those of the specific antibody of interest. The antibody is diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN<sub>3</sub>] into each tube.
- 2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 min. at room temperature (20~25 °C).
- 3) 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.
- 4) Add 40 µL of 1:100 FITC conjugated anti-Hamster IgG (BD Pharmingen; code no. 554011) diluted with the washing buffer. Mix well and incubate for 20 min. at room temperature.
- 5) 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.
- 6) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments, Beckman Coulter; code no. A11895) or OptiLyse B (for analysis on BD instruments, Beckman Coulter; code no. IM-1400), using the procedure recommended in the respective package inserts.
- 7) Add 1 mL of distilled water to each tube and incubate for 10 min. at room temperature.
- 8) Centrifuge at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration.
- 9) Add 1 mL of 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 analysis of Hamster IgG on human PBMC**

Open: Syrian Hamster IgG (M189-3)  
Closed: Secondary antibody alone