

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



# Normal Sheep IgG

|                     |   |
|---------------------|---|
| <b>CODE No.</b>     | PM089   |
| <b>CLONALITY</b>    | Polyclonal  |
| <b>ISOTYPE</b>      | Sheep IgG   |
| <b>QUANTITY</b>     | 100 µL, 5 mg/mL   |
| <b>SOURCE</b>       | Purified IgG from normal sheep serum  |
| <b>REACTIVITY</b>   | No specific reaction was detected on Immunocytochemistry and Flow cytometry                   |
| <b>FORMULATION</b>  | PBS containing 50% glycerol (pH 7.2). 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. |
| <b>APPLICATIONS</b> |   |
|                     | <u>Immunocytochemistry</u>  |
|                     | <u>Flow cytometry</u>   |

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

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



## **RELATED PRODUCTS**

### Purified antibodies

M075-3 Mouse IgG1 (isotype control) (2E12)  
M075-4 Mouse IgG1 (isotype control)-FITC (2E12)  
M075-5 Mouse IgG1 (isotype control)-PE (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)  
M075-8 Mouse IgG1 (isotype control)-Agarose (2E12)  
M075-11 Mouse IgG1 (isotype control)-Magnetic Beads (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-A48 Mouse IgG2a (isotype control)-Alexa Fluor<sup>®</sup> 488 (6H3)  
M076-A64 Mouse IgG2a (isotype control)-Alexa Fluor<sup>®</sup> 647 (6H3)  
M076-11 Mouse IgG2a (isotype control)-Magnetic Beads (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-A48 Mouse IgG2b (isotype control)-Alexa Fluor<sup>®</sup> 488 (3D12)  
M077-A64 Mouse IgG2b (isotype control)-Alexa Fluor<sup>®</sup> 647 (3D12)  
M077-11 Mouse IgG2b (isotype control)-Magnetic Beads (3D12)  
M078-3 Mouse IgG3 (isotype control) (6A3)  
M078-4 Mouse IgG3 (isotype control)-FITC (6A3)  
M079-3 Mouse IgM (isotype control) (7E10)  
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)  
M080-A64 Rat IgG1 (isotype control)-Alexa Fluor<sup>®</sup> 647 (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-A48 Rat IgG2a (isotype control)-Alexa Fluor<sup>®</sup> 488 (2H3)  
M081-A64 Rat IgG2a (isotype control)-Alexa Fluor<sup>®</sup> 647 (2H3)  
M081-8 Rat IgG2a (isotype control)-Agarose (2H3)  
M081-11 Rat IgG2a (isotype control)-Magnetic Beads (2H3)  
M082-3 Rat IgG2c (isotype control) (6E12)  
M082-4 Rat IgG2c (isotype control)-FITC (6E12)  
M090-3 Rat IgG2b (isotype control) (3G8)  
M090-4 Rat IgG2b (isotype control)-FITC (3G8)  
M090-5 Rat IgG2b (isotype control)-PE (3G8)  
M090-A48 Rat IgG2b (isotype control)-Alexa Fluor<sup>®</sup> 488 (3G8)  
M090-A64 Rat IgG2b (isotype control)-Alexa Fluor<sup>®</sup> 647 (3G8)  
PM035 Normal Rabbit IgG (polyclonal)  
PM035-8 Normal Rabbit IgG-Agarose (polyclonal)  
PM067 Normal Guinea Pig IgG (polyclonal)  
M189-3 Syrian Hamster IgG (isotype control)  
M199-3 Armenian Hamster IgG (isotype control)  
PM084 Normal Chicken IgY (polyclonal)  
PM084-4 Normal Chicken IgY-FITC (polyclonal)  
PM084-5 Normal Chicken IgY-PE (polyclonal)  
PM089 Normal Sheep IgG (polyclonal)

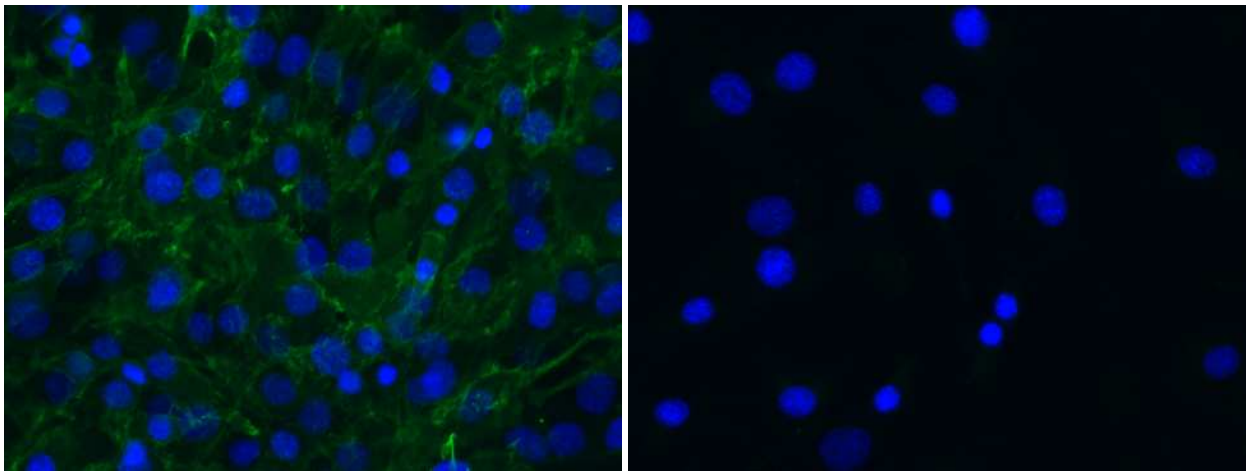
M081-3M2 Rat IgG2a (isotype control) (2H3)  
M090-3M2 Rat IgG2b (isotype control) (3G8)  
M079-3M2 Mouse IgM (isotype control) (7E10)

### Functional grade antibodies

M075-3M2 Mouse IgG1 (isotype control) (2E12)  
M076-3M2 Mouse IgG2a (isotype control) (6H3)  
M077-3M2 Mouse IgG2b (isotype control) (3D12)  
M078-3M2 Mouse IgG3 (isotype control) (6A3)  
M079-3M2 Mouse IgM (isotype control) (7E10)  
M080-3M2 Rat IgG1 (isotype control) (1H5)

### **Immunocytochemistry**

- 1) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 10 min. at room temperature (20~25°C).
- 2) Wash the slide 3 times with PBS.
- 3) Incubate the cells with Normal Sheep IgG (PM089) diluted with PBS containing 2% fetal calf serum (FCS) at the concentration comparable to the specific antibody of interest for 3 hr. at room temperature.
- 4) Wash the slide 3 times with PBS.
- 5) Incubate the cells with 1:100 of Donkey anti-Sheep IgG (H+L) Secondary Antibody, Alexa Fluor® 488 conjugate (ThermoFisher Scientific; code no. A-11015) diluted with PBS containing 2% FCS for 1 hr. at room temperature.
- 6) Visualize by reacting for 5 min. with DAPI.
- 7) Wash the slide 1 time with PBS. Now ready for mounting.



### ***Immunocytochemical detection of M-Cadherin***

Cells: C2C12

Blue: DAPI

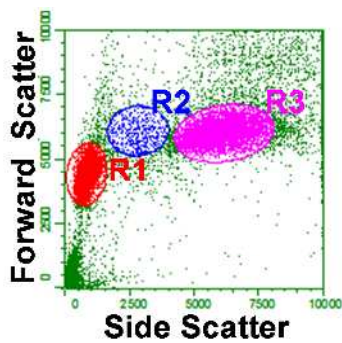
Green:

Left; Human M-Cadherin/Cadherin-15 Antibody (R&D Systems; code no. AF4096), 10 µg/mL

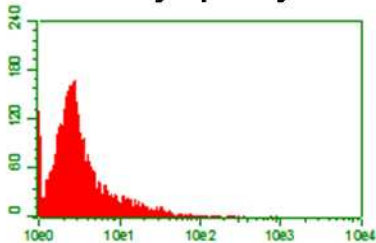
Right; Normal Sheep IgG (PM089), 10 µg/mL

**Flow cytometric analysis for whole blood cells**

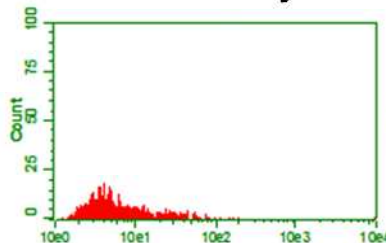
- 1) Dispense 100  $\mu$ L of whole blood into each tube.
- 2) Add 40  $\mu$ L of 5 or 10  $\mu$ g/mL Normal Sheep IgG (PM089) diluted washing buffer [PBS containing 2% fetal calf serum (FCS)]. Mix well and incubate for 30 min. at room temperature.
- 3) Wash the cells 1 time with 1 mL of washing buffer.
- 4) Add 30  $\mu$ L of 1:400 Donkey anti-Sheep IgG (H+L) Secondary Antibody, Alexa Fluor<sup>®</sup> 488 conjugate (ThermoFisher Scientific; code no. A-11015) diluted with washing buffer. Mix well and incubate for 15 min. at room temperature.
- 5) Wash the cells 1 time with 1 mL of washing buffer.
- 6) Add 100  $\mu$ L of OptiLyse B (for analysis on BD instruments, Beckman Coulter; code no. IM-1400). Mix well and incubate for 10 min. at room temperature.
- 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) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.



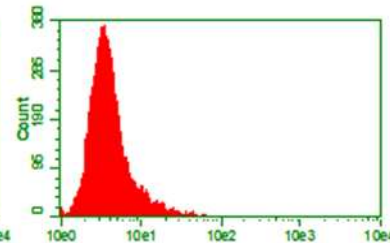
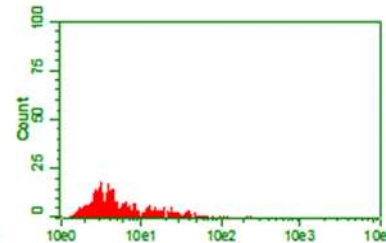
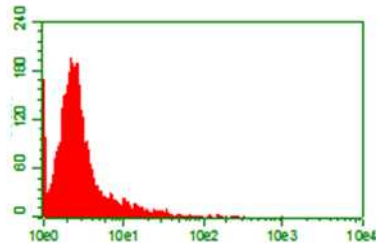
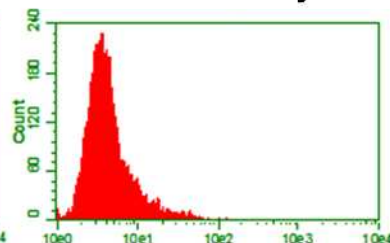
**R1: Lymphocyte**



**R2: Monocyte**



**R3: Granulocyte**

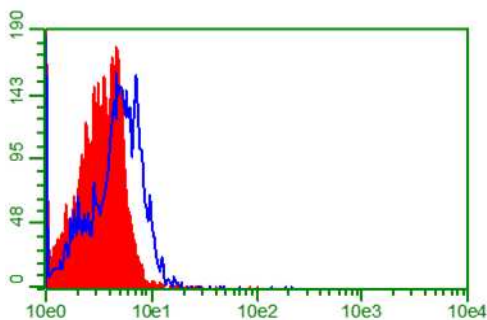


***Flow cytometric analysis of Normal Sheep IgG on human PBMC***

Upper: Normal Sheep IgG (PM089), 5  $\mu$ g/mL  
Lower: Normal Sheep IgG (PM089), 10  $\mu$ g/mL

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

- 1) Wash C2C12 cells ( $2.5 \times 10^5$  cells/sample) 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 2) Fix the cells with PBS containing 4% paraformaldehyde (PFA) for 10 min. at room temperature.
- 3) Wash the cells 2 times with 1 mL of washing buffer (+) [PBS containing 2% fetal calf serum (FCS) 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.
- 4) Add 10  $\mu$ 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 10 min. at room temperature.
- 5) Add 50  $\mu$ L of Normal Sheep IgG (PM089) diluted with washing buffer (+) at the concentration comparable to the specific antibody of interest. Mix well and incubate for 3 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 6) Wash the cells 1 time with 1 mL of washing buffer.
- 7) Add 40  $\mu$ L of 1:400 Donkey anti-Sheep IgG (H+L) Secondary Antibody, Alexa Fluor<sup>®</sup> 488 conjugate (ThermoFisher Scientific; code no. A-11015) diluted with washing buffer. Mix well and incubate for 1 hr. at room temperature.
- 8) Wash the cells 1 time with 1 mL of washing buffer.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.



### ***Flow cytometric analysis of Normal Sheep IgG on C2C12 cells***

Open: Human M-Cadherin/Cadherin-15 Antibody (R&D Systems; code no. AF4096), 20  $\mu$ g/mL  
Closed: Normal Sheep IgG (PM089), 20  $\mu$ g/mL