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

MBL
A JSR Life Sciences Company

Armenian Hamster IgG (isotype control)

CODE No. M199-3

CLONALITY Monoclonal
CLONE ttko2
ISOTYPE Armenian Hamster IgG
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant using protein A agarose.
REACTIVITY No specific reaction was detected on immunoprecipitation and flow cytometry.
FORMURATION 1 mg/mL in PBS containing 50% glycerol. 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

Immunoprecipitation

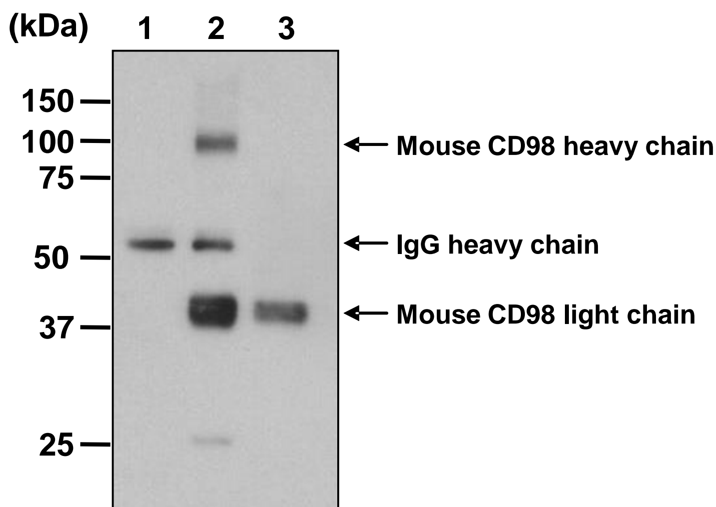
Flow cytometry

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 https://ruo.mbl.co.jp/.

Immunoprecipitation

1) Wash 1 x 10^7 cells twice 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 1 hr. at room temperature.
4) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 400 µL 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 250 µL 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 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge for 5 min.
9) Load 10 µL 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^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
13) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) [5 min. x 3 times].
14) Incubate the membrane with 1:1,000 of goat anti-Armenian hamster IgG-HRP (Santa Cruz; code no. SC-2443) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
15) Wash the membrane with PBS-T (5 min. x 3 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.

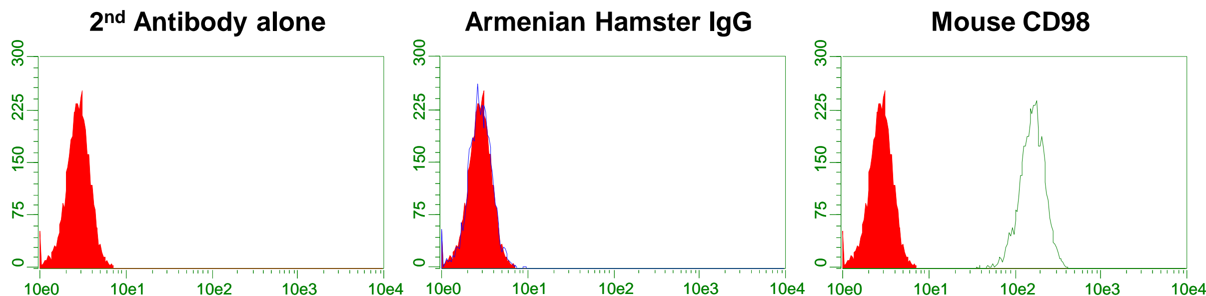


Immunoprecipitation of mouse CD98 from P3U1 cells

Lane 1: Armenian Hamster IgG (M199-3)
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

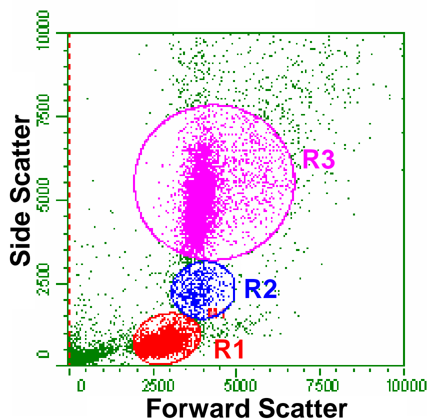
1) Wash the cells once with 1 mL of the washing buffer [PBS containing 2% fetal calf serum (FCS)].
2) Add 200 μL of 4% paraformaldehyde (PFA)/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature (20~25°C).
3) Wash the cells twice with 1 mL of the washing buffer.
4) Add 20 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 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 15 min. at room temperature.
6) Wash the cells 1 time with 1 mL of the washing buffer.
7) Add 40 μL of 10 μg/mL FITC conjugated anti-Hamster IgG (BD Pharmingen; code no. 554011) diluted with the washing buffer. Mix well and incubate for 15 min. at room temperature.
8) Wash the cells 1 time with 1 mL of the washing buffer.
9) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.



Flow cytometric analysis of mouse CD98 expression on P3U1 cells
Open: Armenian Hamster IgG (M199-3) or mouse CD98 (D137-3)
Closed: Secondary antibody alone

Flow cytometric analysis for whole blood cells

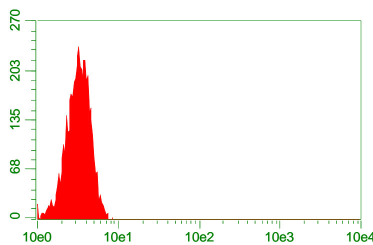
1) Add the isotype control antibody at the concentrations comparable to those of the specific antibody of interest. The antibody is diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3] into each tube.
2) Add 100 µL of whole blood into each tube. Mix well, and incubate for 20 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 the 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.

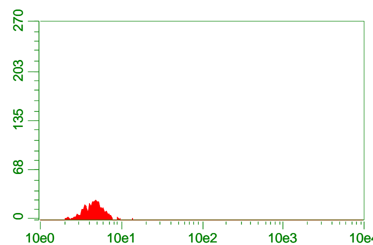


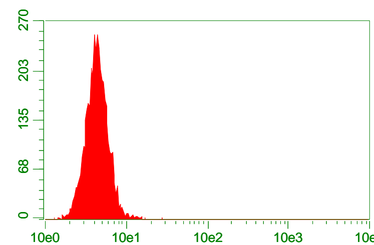
R1: Lymphocyte

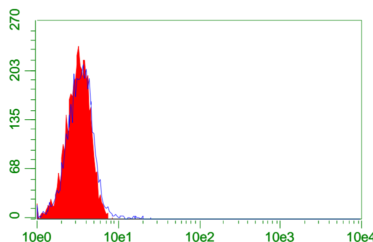
R2: Monocyte

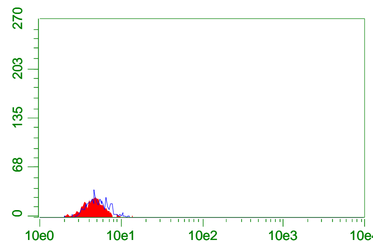
R3: Granulocyte

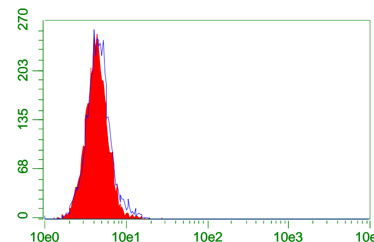












Flow cytometric analysis of Hamster IgG on human PBMC

Open: Armerian Hamster IgG (M199-3)
Closed: Secondary antibody alone