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

MONOCLONAL ANTIBODY

Mouse CD61

Code No.	Clone	Subclass	Quantity	Concentration
M109-3	1-55-4	Rat IgG2a κ	100 µg	1 mg/mL

BACKGROUND: Platelet activation results in a conformational change of the membrane spanning platelet integrin GPIIb/IIIa (αIIbβ3, CD41/CD61) enabling the binding of the plasma protein fibrinogen. This binding is primarily reversible, but it enhances platelet activation by outside-in signal causing receptor clustering, platelet secretion, and finally irreversible fibrinogen binding and platelet aggregation.

SOURCE: This antibody was purified from hybridoma (clone 1-55-4) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Wister rat lymphocyte immunized with mouse platelet.

FORMULATION: 100 µg IgG in 100 µL volume of 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.

REACTIVITY: This antibody reacts with mouse CD61 antigen on Western blotting, Immunoprecipitation and Flow cytometry.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; 10 µg/200 µL of cell extract from 5 x 10^6 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	PBMC	bone marrow	Not Tested
Reactivity on FCM	-	+	

INTENDED USE:

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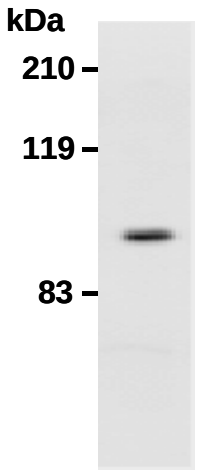
REFERENCES:

1) Schwarz, M., *et al.*, *J. Pharmacol. Exp. Ther.* **308**, 1002-1011 (2004)
2) De Nichilo, M. O., *et al.*, *J. Immunol.* **156**, 284-288 (1996)

RELATED PRODUCTS:

D202-3 mouse CD11b (1C4)
D202-4 FITC labeled mouse CD11b (1C4)
M100-3 mouse CD11c (223H7)
M100-4 FITC labeled mouse CD11c (223H7)
M100-6 Biotin labeled mouse CD11c (223H7)
M109-3 mouse CD61 (1-55-4)
K0046-3 Anti-mouse Integrin α7 (3C12)
K0046-5 PE labeled anti-mouse Integrin α7 (3C12)
K0047-3 Anti-mouse Integrin α7 (6A11)
M081-3 Rat IgG2a Isotype control (2H3)
M081-4 FITC labeled Rat IgG2a Isotype control (2H3)
M081-8 Agarose conjugated Rat IgG2a Isotype control (2H3)



Western blot analysis mouse CD61 expression in mouse platelet using M109-3 on the non-reduced condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the mouse platelets 3 times with PBS
2) Mix the sample with equal volume of Laemmli's sample buffer (without 2-mercaptoethanol).
3) Boil the samples for 5 minutes and centrifuge. Load 15 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.

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URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

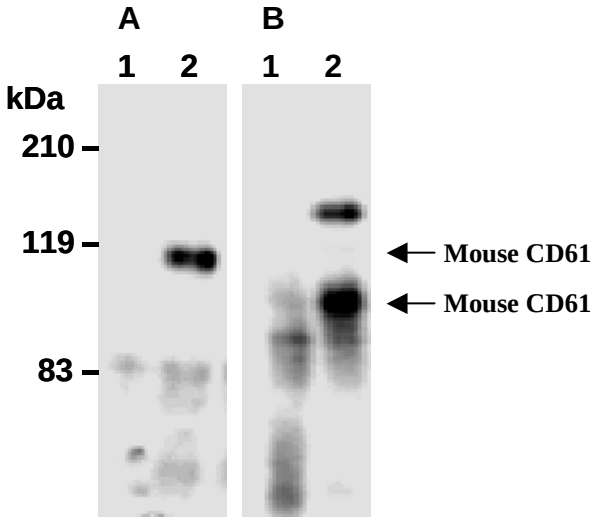
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 °C.
6) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
8) Incubate the membrane with the 1:2,000 HRP-conjugated anti-rat IgG (MBL; code no. IM-0825) 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 6 times).
10) Wipe excess buffer on the membrane, 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 5 minutes. Develop the film as usual. The condition for exposure and development may vary. Expose to an X-ray film in a dark room for 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for western blotting; mouse platelet)

Immunoprecipitation

1) Wash the biotin labeled mouse bone marrow cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (20 mM Tris-HCl pH 8.0, 250 mM NaCl, 0.25% NP-40, 1 mM EDTA, 1 mM DTT) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 60 minutes.
2) Centrifuge the tube at 800 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 1 mL of the supernatant. Mix well and incubate with gentle agitation for 1 hour at 4°C.
4) Add 20 μL of 50% protein G agarose beads resuspended in the cold PBS. Mix well and incubate with gentle agitation for 1 hour at 4°C.
5) Wash the beads 3-5 times with the cold washing buffer (10 mM Tris-HCl pH 8.0, 500 mM NaCl, 0.1% NP-40) (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 μL of Laemmli's sample buffer (with or without 2-mercaptoethanol) and boil the samples for 5 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
7) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
8) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 °C.
9) Incubate the membrane with the 1:2,0000 HRP-conjugated streptavidin (MBL; code no. IM-0309) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS](5 minutes x 6 times).
11) Wipe excess buffer on the membrane, 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.
12) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; mouse bone marrow cell)



Immunoprecipitation of mouse CD61 from mouse bone marrow cells with rat IgG2a (1) or M109-3 (2) on the reduced condition (A) and the non-reduced condition (B). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with HRP-Streptavidin.*

Flow cytometric analysis for floating cells

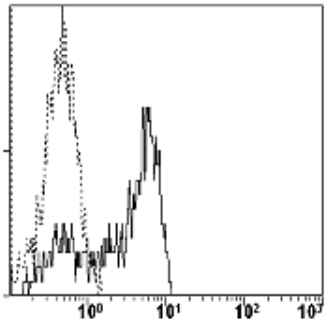
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 μL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN_3 to the cell pellet after

tapping. Mix well and incubate for 5 minutes at room temperature.

5) Add 40 μL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) 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.
7) Add 30 μL of 1:40 PE conjugated anti-rat IgG (MBL; code no. IM-1623) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
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.
9) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; mouse bone marrow cells)



Flow cytometric analysis of mouse CD61 expression on mouse bone marrow cells. *Dotted line indicates the reaction of isotypic control to the cells. Solid line indicates the reaction of M109-3 to the cells.*