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Product datasheet for AM26637AF-N

CD61 / ITGB3 Rat Monoclonal Antibody [Clone ID: 1-55-4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	1-55-4
Applications:	FC, IP, WB
Recommended Dilution:	Western blot: 1 µg/ml for chemiluminescence detection system. Immunoprecipitation: 10 µg/200 µl of cell extract from 5 x 10^6 cells. Flow cytometry: 10 µg/ml (final concentration). For details see protocols below.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Mouse platelet
Specificity:	This antibody reacts with CD61 antigen.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein G agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	integrin beta 3
Database Link:	Entrez Gene 16416 Mouse O54890

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Background: Platelet activation results in a conformational change of the membrane spanning platelet integrin GPIIb/IIIa (aIIbß3, 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.

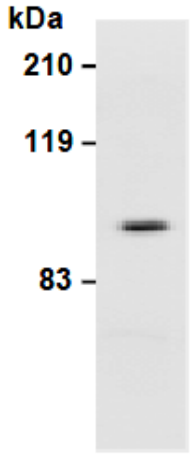
Synonyms: Integrin beta-3, GP3A, GPIIIa

Note: This product was originally produced by MBL International.

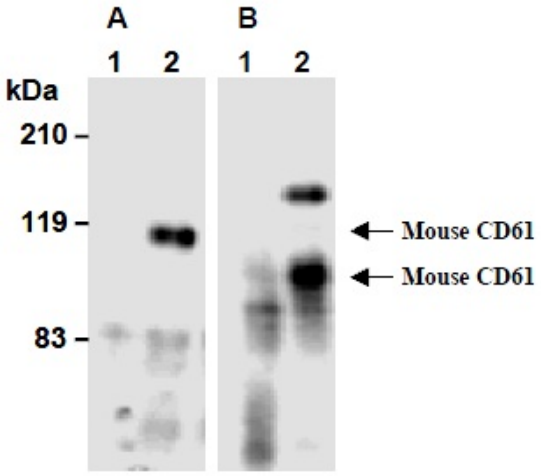
Protocol:
SDS-PAGE & Western Blotting
1) Wash the mouse platelets 3 times with PBS
2) Mix the sample with equal volume of Laemmli's sample buffer (without 2-mercaptoethanol).
3) Boil the samples for 5 minutes and centrifuge. Load 15 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 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 oC.
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 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 4oC with rotating for 60 minutes.
2) Centrifuge the tube at 800 x g for 10 minutes at 4oC 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 4oC.
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 4oC.
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/cm2 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 oC.
9) Incubate the membrane with the 1:2,0000 HRP-conjugated streptavidin 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)
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% NaN3].
2) Resuspend the cells with washing buffer (5x10e6 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 (20~25oC). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 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 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)

Product images:



Western blot analysis mouse CD61 expression in mouse platelet using AM26637AF-N on the non-reduced condition.



Immunoprecipitation of mouse CD61 from mouse bone marrow cells with rat IgG2a□(1)□or AM26637AF-N (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.