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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-Aggrus (Podoplanin) (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D190-3	8F11	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: In addition to hemostasis and host defense, platelets are involved in the induction of inflammation, tissue repair, and tumor metastasis. Aggrus (T1α/Podoplanin) is a novel 36-45 kDa membrane sialoglycoprotein that induces platelet aggregation. Aggrus is highly expressed in various tumor cells and the platelet aggregation ability of Aggrus depends on specific oligosaccharide structures that may be related to the metastasis process in cancer cells. Aggrus has been identified as a diagnostic tumor marker for seminomas and testicular cancers.

SOURCE: This antibody was purified from hybridoma (clone 8F11) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X63-Ag8U1 with Sprague-Dawle rat splenocyte immunized with NL-17 cell membrane fraction.

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 Aggrus (Podoplanin) (44 kDa) on Western blotting, Immunoprecipitation and Flow cytometry.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; 1-2 µg/250 µL of cell extract from 2.5 x 10^6 cells
Flow cytometry; 5-10 µg/mL (final concentration)
Immunohistochemistry; 1.25 µg/mL (whole mount)
Immunocytochemistry; Not tested*
*It is reported that this monoclonal antibody can be used in Immunocytochemistry in the reference number 2).

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Not tested	Transfectant, NL-17	Not tested
Reactivity on WB	-*	+	

*The reference 7) described that this antibody does not react to human Aggrus (Podoplanin).

REFERENCES:

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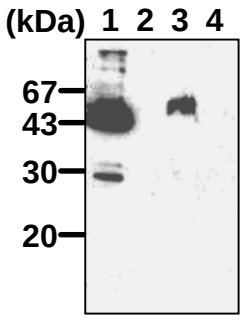
2) Fu, J., *et al., J. Clin. Invest.* **118**, 3725-3737 (2008) [IC, IHC]
3) Kato, Y., *et al., Cancer Sci.* **99**, 54-61 (2008)
4) Kunita, A., *et al., Am. J. Pathol.* **170**, 1337-1347 (2007)
5) Katayama, K., *et al., Mol. Cell. Biol.* **25**, 5725-5737 (2005)
6) Kaneko, M., *et al., J. Biol. Chem.* **279**, 38838-38843 (2004)
7) Kato, Y., *et al., J. Biol. Chem.* **278**, 51599-51605 (2003)
8) Watanabe, M., *et al., Cancer Res.* **50**, 6657-6662 (1990)
9) Watanabe, M., *et al., Cancer Res.* **48**, 6411-6416 (1988)

Clone 8F11 is used in these references.



Western blot analysis of mouse Aggrus (Podoplanin)

Lane 1: mouse Aggrus (Podoplanin)/CHO
Lane 2: parental cell (CHO)
Lane 3: NL-17
Lane 4: NL-14
Immunoblotted with D190-3

Samples were kindly provided by Dr. Naoya Fujita. (Institute of Molecular and Cellular Biosciences, The University of Tokyo)

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

SDS-PAGE & Western Blotting

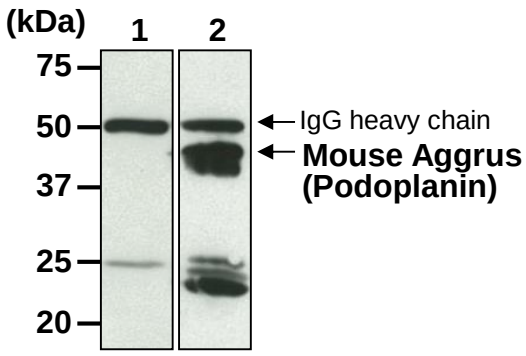
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) 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.
6) 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.
7) 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.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with 1:10,000 Anti-IgG (Rat) pAb-HRP (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with 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 controls for Western blotting; Transfectant and NL-17)

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 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 250 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 μL of 50% protein G agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 μL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; NL-17)



Immunoprecipitation of mouse Aggrus (Podoplanin)

Sample: NL-17
Lane 1: Rat IgG2a (isotype control) (M081-3)
Lane 2: D190-3
Immunoblotted with D190-3

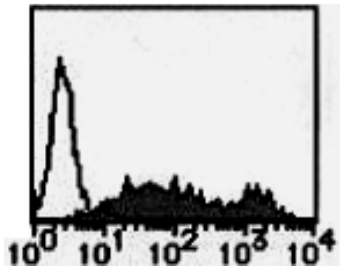
Sample was kindly provided by Dr. Naoya Fujita. (Institute of Molecular and Cellular Biosciences, The University of Tokyo)

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 1:100 Anti-IgG (Rat) pAb-FITC (MBL code no. IM-0827) 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; Transfectant)



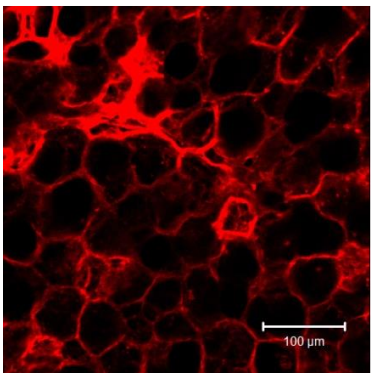
Flow cytometric analysis of mouse Aggrus (Podoplanin)/CHO

Closed: D190-3
Open: Isotype control (M081-3)

Sample was kindly provided by Dr. Naoya Fujita. (Institute of Molecular and Cellular Biosciences, The University of Tokyo)

Immunohistochemical staining for paraffin-embedded sections (whole mount)

1) Inflate alveoli of the lung of euthanized mice by infusing 2% low melting agar.
2) Collect the left lobe and fix with 4% paraformaldehyde for 10 minutes at 4°C, then 20 minutes at room temperature.
3) Cut the left lobe into 2-mm cube pieces and further fix with 4% paraformaldehyde for 1 hour at 4°C.
4) Soak and wash the specimen with washing buffer (0.1% Triton X-100 in PBS) for 30 minutes at room temperature.
5) Exchange the washing buffer to blocking buffer (5% BSA in washing buffer) and keep the specimen for overnight at 4°C.
6) Add the specimen with the primary antibody diluted with the blocking buffer at the concentration indicated in **APPLICATIONS** and incubated for 18 hours at 4°C.
7) Wash the specimen with 1% BSA in the washing buffer 3 times for 30 minutes each at room temperature.
8) Add 1:200 of anti-rat IgG-Qdot®605 (Invitrogen; code no. Q11601MP) diluted with the blocking buffer for 2 hours at room temperature.
9) Wash the specimen with 1% BSA in washing buffer 3 times for 30 minutes each at room temperature.
10) Immerse and equilibrate the specimen with 50% glycerol in PBS.



Immunohistochemical detection (whole mount) of mouse Aggrus (Podoplanin) on paraffin embedded section of mouse normal lung with D190-3

This data was kindly provided by Dr. Harayama and Dr. Shindou, The University of Tokyo

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