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

MONOCLONAL ANTIBODY

Anti-Human Aggrus/Podoplanin

Code No.	Clone	Subclass	Quantity
D189-1	YM-1	Rat IgG2a	100 µL

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 seminoma.

SOURCE: This antibody was concentrated from hybridoma (clone YM-1) supernatant. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Sprague-Dawley rat splenocyte immunized with human Aggrus N-terminal peptide (38-51aa.).

FORMULATION: 100 µL aliquot of concentrate antibody from the supernatant with preservative (0.09% sodium azide).
*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.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with human Aggrus (36 kDa) on Western blotting.

APPLICATIONS:
Western blotting; 1:500 for chemiluminescence detection system
Immunoprecipitation; Not determined
Immunohistochemistry; 1:20
Immunocytochemistry; Not tested
Flow cytometry; 1:25

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	LEC, Transfectant	NL-17, Transfectant	Transfectant
Reactivity on WB	+	-	-

(LEC: Lymphatic Endothelial Cells)

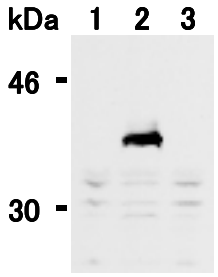
INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

REFERENCES:
1) Kunita, A., *et al.*, *Am. J. Pathol.* **170**, 1337-1347 (2007)
2) Kaneko, M., *et al.*, *J. Biol. Chem.* **279**, 38838-38843 (2004)
3) Kato, Y., *et al.*, *Oncogene* **23**, 8552-8556 (2004)
4) Kato, Y., *et al.*, *J. Biol. Chem.* **278**, 51599-51605 (2003)

Clone YM-1 is used in reference number 1) and 2).

RELATED PRODUCTS:

D190-3	Anti-Mouse Aggrus/Podoplanin (8F11)
M084-3	Anti-human Podocalyxin/PCLP1 (53D11)
M084-4	FITC labeled anti-human Podocalyxin/PCLP1 (53D11)
M085-3	Anti-human Podocalyxin/PCLP1 (4H11)
D072-3	Anti-mouse Podocalyxin/PCLP1 (10B9)
D072-4	FITC labeled anti-mouse Podocalyxin/PCLP1 (10B9)
D072-5	PE labeled anti-mouse Podocalyxin/PCLP1 (10B9)
D072-6	Biotin labeled anti-mouse Podocalyxin/PCLP1 (10B9)

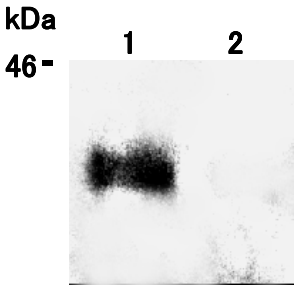


Western blot analysis of human Aggrus/Podoplanin expression in mock transfected CHO (1), human Aggrus/Podoplanin transfected CHO (2) and mouse Aggrus/Podoplanin transfected CHO cells (3) using D189-1.

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 cold 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 4~20% gradient gel (Daiichi Pure Chemicals) for electrophoresis.
5) Blot the protein to a nitrocellulose membrane at 50 V for 16 hour at 4°C 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 blocking buffer [4% skimmed milk in 25 mM Tris (pH 8.0), 125 mM NaCl, 0.1% Tween 20] for 2 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with blocking buffer as suggest in the **APPLICATIONS** for 2 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with blocking buffer (15 minutes x 3 times).
9) Incubate the membrane with the 1:500 HRP-conjugated anti-rat IgG (Amershambioscience) diluted with blocking buffer for 1 hour at room temperature.
10) Wash the membrane with washing buffer [25 mM Tris (pH 8.0), 125 mM NaCl, 0.025% Tween-20] (5 minutes x 3 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 10 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; transfectant, LEC)



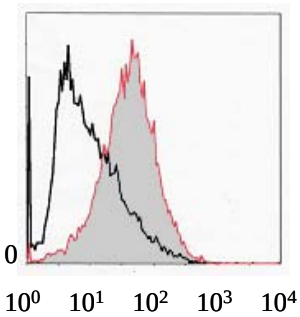
Western blot analysis of human Aggrus/Podoplanin expression in LEC (1) and mock transfected CHO cells (2) using D189-1.

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 the washing buffer [PBS (Ca^{2+}, Mg^{2+} free)].
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 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.
5) 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.
6) Add 30 μL of 1:200 FITC conjugated anti-rat IgG (Cappel) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
7) 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.
8) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LEC)



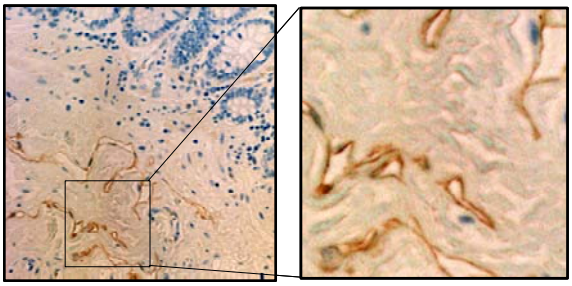
Flow cytometric analysis of human Aggrus/Podoplanin expression on LEC. *Open histogram indicates the reaction of isotypic control to LEC. Shaded histogram indicates the reaction of D189-1 to LEC.*

Immunohistochemical staining for paraffin embedded sections: ABC method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 2 times for 3-5 minutes each.
3) Wash the slides with 95% Ethanol 2 times for 3-5 minutes each.
4) Wash the slides with deionized water for 30 seconds.

5) Remove the slides from the deionized water and cover each section with 3% H_2O_2 for 5 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Biotin labeled anti-rat IgG (DakoCytomation; code no. E0468). Incubate for 15 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (LSAB+System-HRP Kit: DakoCytomation; code no. K0679). Incubate for 15 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 5 minutes with substrate solution containing 7.5 mg DAB, 40 μL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 2-5 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
13) Now ready for mounting.

(Positive control for Immunohistochemistry; human normal rectum)



Immunohistochemical detection of human Aggrus/Podoplanin on paraffin embedded section of human normal rectum with D189-1.

These data were gladly provided by Dr. N. Fujita, Ph.D. and Dr. Y. Kato, M.D., Ph.D. in Department of Cell Growth and Regulation, Institute of Molecular and Cellular Biosciences in the University of Tokyo.