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

Anti-Podoplanin (Mouse) mAb

CODE No. D321-3

CLONALITY Monoclonal
CLONE PMab-1
ISOTYPE Rat IgG2a κ
QUANTITY 100 µL, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
FORMULATION 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.

APPLICATIONS-CONFIRMED

Western blotting	1 µg/mL
Immunoprecipitation	1 µg/10 µg of tissue and cell lysate
Immunocytochemistry	Can be used*
Immunohistochemistry	Can be used*
Flow cytometry	1 µg/mL

*verified in development only; not validated per production lot. Please use it at your own discretion.

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cell	Transfectant	Transfectant, NL-17, NL-14	Transfectant	Transfectant
Reactivity	–	+	–	–

Entrez Gene ID 14726 (Mouse)

REFERENCES

1) Kaji, C., *et al.*, *Acta. Histochem. Cytochem.* in press
2) Kato, Y., *et al.*, *Biochem. Biophys. Res. Commun.* **349**, 1301-1307 (2006)
3) Kaneko, M. K., *et al., FEBS Lett.* **581**, 331-336 (2007)
4) Kato, Y., *et al.*, *Cancer. Sci.* **99**, 54-61 (2008)
5) Ogasawara, S., *et al.*, *Hybridoma* **27**, 259-267 (2008)
6) Kato, Y., *et al.*, *Nucl. Med. Biol.* **37**, 785-794 (2010)

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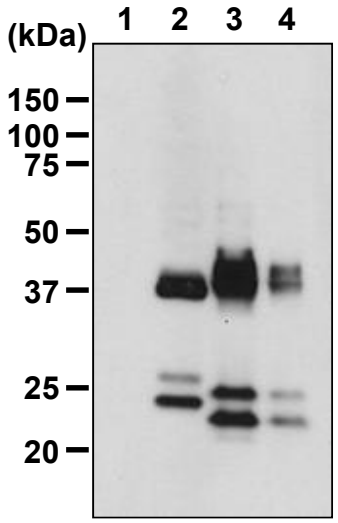
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Boil the samples for 3 min. and centrifuge. Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
4) 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% MeOH). See the manufacturer's manual for precise transfer procedure.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
8) Incubate the membrane with the 1:10,000 anti-IgG (Rat)-HRP (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times).
10) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min. 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 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; transfectant, NL-17, NL-14)

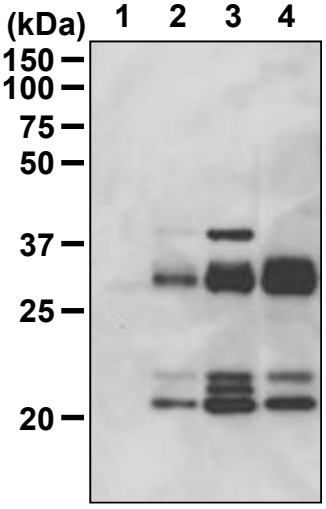


Western blot analysis of mouse Podoplanin
Lane 1: Parental cell (CHO)
Lane 2: mouse Podoplanin/CHO
Lane 3: NL-17
Lane 4: NL-14
Immunoblotted with D321-3

Immunoprecipitation

1) Wash 5 x 10^6 cells 2 times 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, then sonicate briefly (up to 15 sec.). Then, incubate for 15 min. on ice.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Mix 20 µL of 50% protein G agarose beads slurry resuspended in 250 µL of IP buffer (10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gently agitation for 1 hr. at room temperature. (The amount of antibody will depend on the conditions.)
4) Wash the beads 3 times with 1 mL of IP buffer.
5) Add 250 µL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hr. at room temperature.
6) Wash the beads 6 times with 1 mL of Lysis buffer.
7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
8) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
9) 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% MeOH). See the manufacturer's manual for precise transfer procedure.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for overnight at 4°C.
11) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
12) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
13) Incubate the membrane with the 1:10,000 anti-IgG (Rat)-HRP (MBL; code no. IM-0825) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
14) Wash the membrane with PBS-T (5 min. x 3 times).
15) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
16) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
17) 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.

(Positive control for Immunoprecipitation; transfectant)



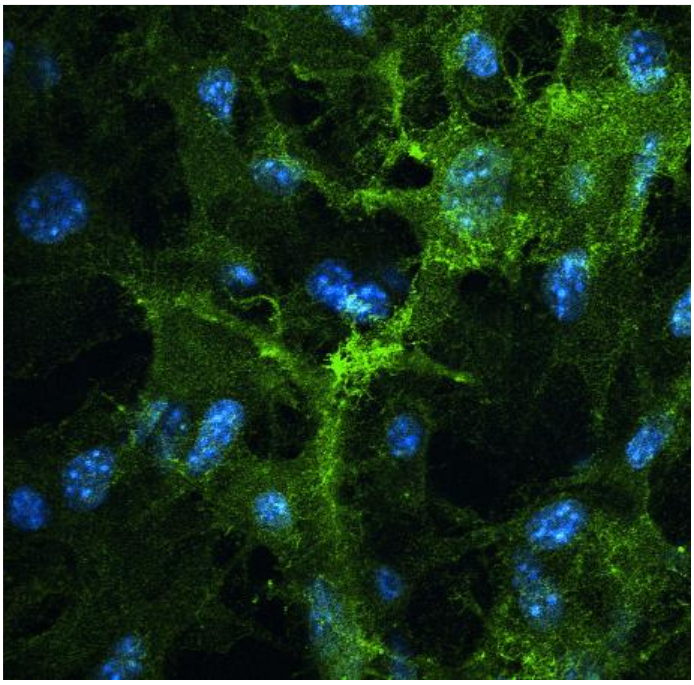
Immunoprecipitation of mouse Podoplanin from CHO transfectant

Lane 1: IP with isotype control (M081-3, 1 µg)
Lane 2: IP with D321-3, 0.5 µg
Lane 3: IP with D321-3, 1 µg
Lane 4: mouse Podoplanin/CHO, whole cell lysate
Immunoblotted with D321-3

Immunocytochemistry

1) Spread the cells on a glass slide, then incubate in a CO_2 incubator for one night.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 5 min. at room temperature (20~25ºC).
4) Wash the slide 2 times in PBS.
5) Add blocking buffer (0.1% normal goat serum /PBS) to the cell and incubate for 30 min. at room temperature.
6) Wash the slide 1 time in PBS.
7) Add 200 µL of the primary antibody diluted with blocking buffer onto the cells and incubate for 8 hr. at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the slide 1 time in PBS for 30 min.
9) Add 100 µL of 0.1 µg/mL anti-IgG (Rat)-Alexa Fluor®488 (Invitrogen; code no. A11006) diluted with blocking buffer onto the cells. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
10) Wash the slide 1 time in PBS for 30 min.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Counter stain with DAPI for 5 min. at room temperature.
13) Wash the slide 1 time in PBS for 5 min.
14) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; B16-F10)

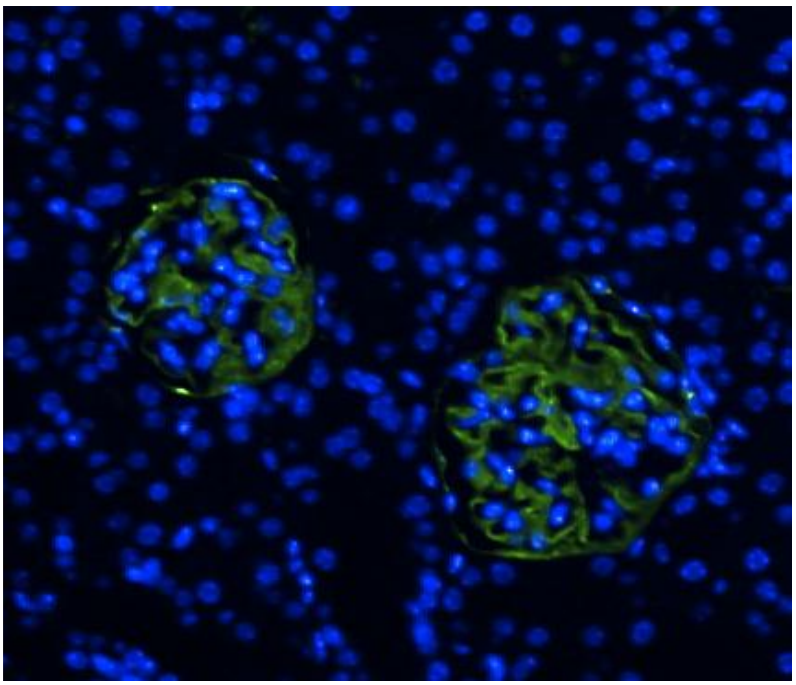


Immunocytochemical detection of mouse Podoplanin in B16-F10
Green: D321-3
Blue: DAPI

Immunohistochemical staining for formalin fixed paraffin-embedded section

1) Deparaffinize the sections with Xylene 3 times for 3 min. each.
2) Wash the slides with Ethanol 3 times for 3 min. each.
3) Wash the slides with PBS 3 times for 3 min. each.
4) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer (0.1% normal goat serum /PBS) for 30 min. at room temperature (20~25ºC) to block non-specific staining. Do not wash.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with blocking buffer for 8 hr. at 4°C. (The concentration of antibody will depend on the conditions.)
6) Wash the slides 3 times in PBS for 5 min. each.
7) Wipe gently around each section and cover tissues with 100 µL of 0.1 µg/mL anti-IgG (Rat)-Alexa Fluor®488 (Invitrogen; code no. A11006) diluted with blocking buffer. Incubate for 30 min. at room temperature. Keep out light by aluminum foil.
8) Wash the slides 3 times in PBS for 5 min. each.
9) Counter stain with DAPI for 5 min. at room temperature.
10) Wash the slide 1 time in PBS for 5 min.
11) Now ready for mounting.

(Positive control for Immunohistochemistry; mouse kidney)

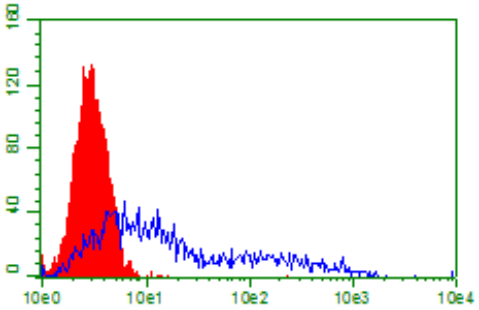


Immunohistochemical detection of mouse Podoplanin in mouse kidney
Green: D321-3
Blue: DAPI

Flow cytometric analysis

1) Wash the cells (5 x 10^5 cells/sample) 3 times with 1 mL of washing buffer (PBS containing 2% fetal calf serum (FCS)).
2) Add 100 µL of 4% paraformaldehyde (PFA)/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
3) Wash the cells 3 times with 1 mL of washing buffer.
4) Add 10 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 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 20 min. at room temperature.
6) Wash the cells 1 time with 1 mL of washing buffer.
7) Add 40 µL of 1:400 anti-IgG (Rat)-Alexa Fluor®488 (Invitrogen; code no. A11006) diluted with the washing buffer. Mix well and incubate for 20 min. at room temperature.
8) Wash the cells 1 time with 1 mL of washing buffer.
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 detection of mouse Podoplanin in CHO transfectant

Open: D321-3
Closed: isotype control (M081-3)

The data of IC and IHC were kindly provided from Dr. Yukinari Kato (Yamagata University, Japan)