

Anti-Podoplanin (Mouse) mAb

CODE No.	D321-3
CLONALITY	Monoclonal
CLONE	PMab-1
ISOTYPE	Rat IgG2a κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
FORMURATION	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

<u>Western blotting</u>	1 μ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	1 μ g/10 μ g of tissue and cell lysate
<u>Immunocytochemistry</u>	0.5 μ g/mL
<u>Immunohistochemistry</u>	0.25-1 μ g/mL
<u>Flow cytometry</u>	1 μ g/mL

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)

For more information, please visit our web site <https://ruo.mbl.co.jp/>

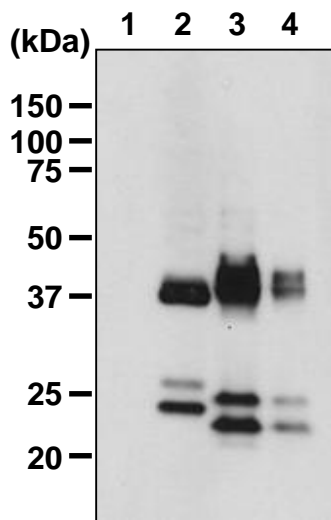
RELATED PRODUCTS

- D320-3 anti-Podoplanin (Human) mAb (NZ-1.2)
- D189-1 anti-Aggrus (Podoplanin) (Human) mAb
(YM-1)
- D190-3 anti-Aggrus (Podoplanin) (Mouse) mAb
(8F11)
- D190-4 anti-Aggrus (Podoplanin) (Mouse) mAb-FITC
(8F11)

SDS-PAGE & Western blotting

- 1) Wash 1×10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
- 2) Centrifuge the tube at $12,000 \times g$ 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 $1 \text{ mA}/\text{cm}^2$ 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)



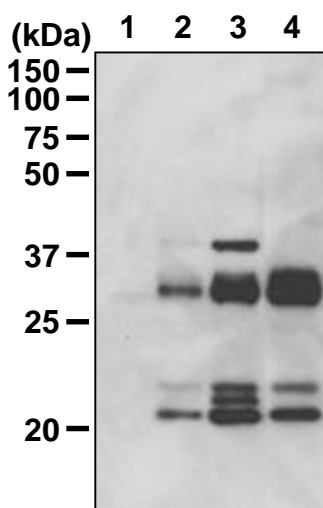
Western blot analysis of mouse Podoplanin

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 5×10^6 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 $12,000 \times g$ 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 $1 \text{ mA}/\text{cm}^2$ 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. \times 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. \times 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)



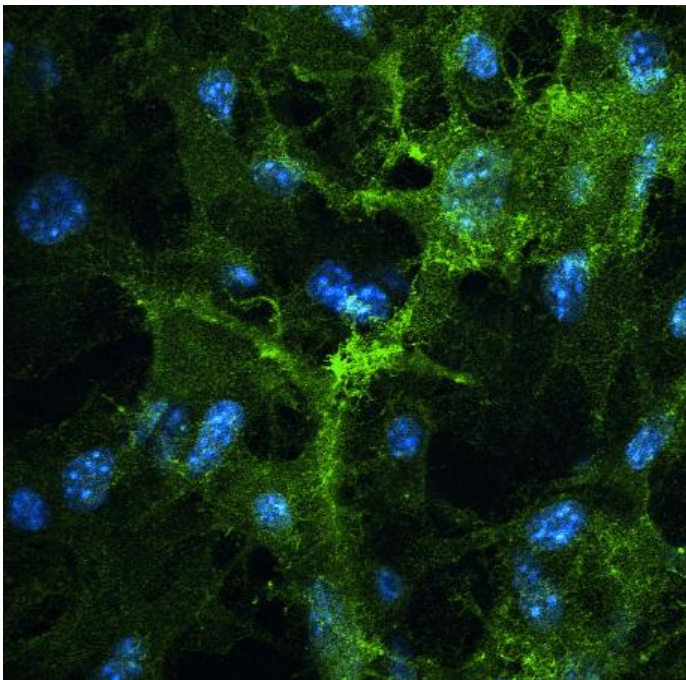
Immunoprecipitation of mouse Podoplanin from CHO transfectant

- 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 CO₂ 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 (20~25°C).
- 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 as suggested in the **APPLICATIONS** 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)



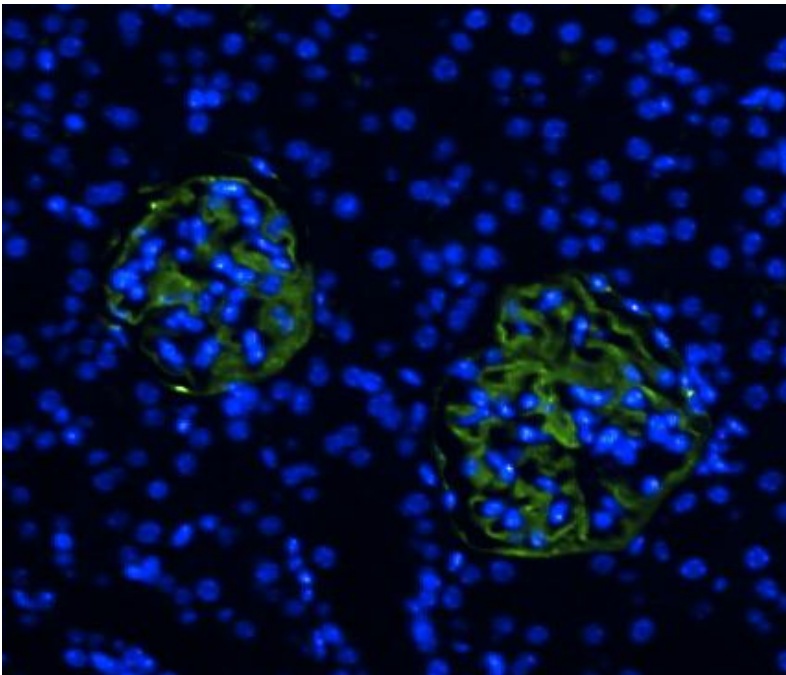
Immunocytochemical detection of mouse Podoplanin in B16-F10

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 (20~25°C) 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 as suggested in the **APPLICATIONS** 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)



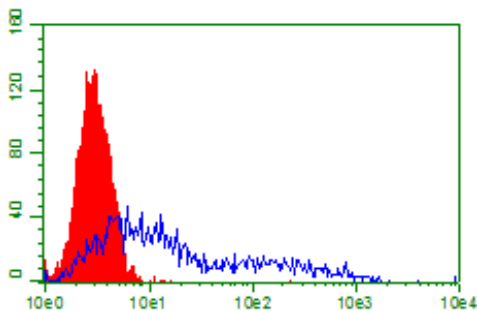
Immunohistochemical detection of mouse Podoplanin in mouse kidney

Green: D321-3
Blue: DAPI

Flow cytometric analysis

- 1) Wash the cells (5×10^5 cells/sample) 3 times with 1 mL of washing buffer (PBS containing 2% fetal calf serum (FCS)).
- 2) Add 100 μ L 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 μ L 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 μ L 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 μ L 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 μ L 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)