

# Anti-Podoplanin (Mouse) mAb

<b>CODE No.</b>	D321-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	PMab-1
<b>ISOTYPE</b>	Rat IgG2a $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	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 $\mu$ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	1 $\mu$ g/10 $\mu$ g of tissue and cell lysate
<u>Immunocytochemistry</u>	0.5 $\mu$ g/mL
<u>Immunohistochemistry</u>	0.25-1 $\mu$ g/mL
<u>Flow cytometry</u>	1 $\mu$ 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)

## RELATED PRODUCTS

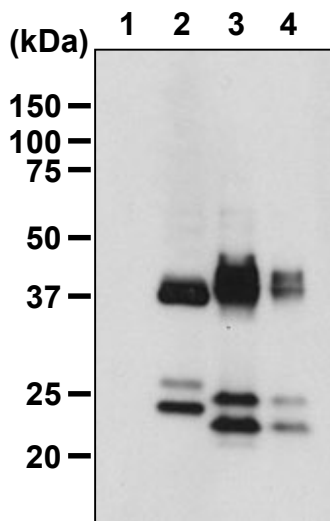
For more information, please visit our web site <https://ruo.mbl.co.jp/>.

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

### **SDS-PAGE & Western blotting**

- 1) Wash  $1 \times 10^7$  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 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  $\mu$ L 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<sup>2</sup> 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)



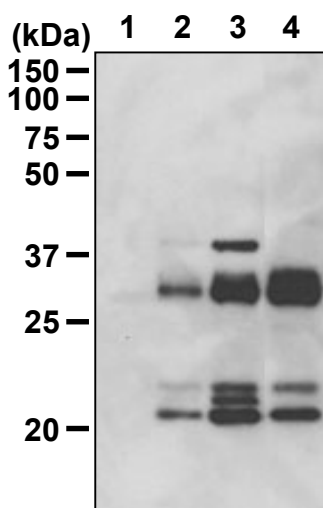
#### ***Western blot analysis of mouse Podoplanin***

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  $5 \times 10^6$  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  $4^\circ\text{C}$  and transfer the supernatant to another tube.
- 3) Mix 20  $\mu\text{L}$  of 50% protein G agarose beads slurry resuspended in 250  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  of Laemmli's sample buffer, boil for 3 min. and centrifuge.
- 8) Load 10  $\mu\text{L}$  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  $4^\circ\text{C}$ .
- 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)



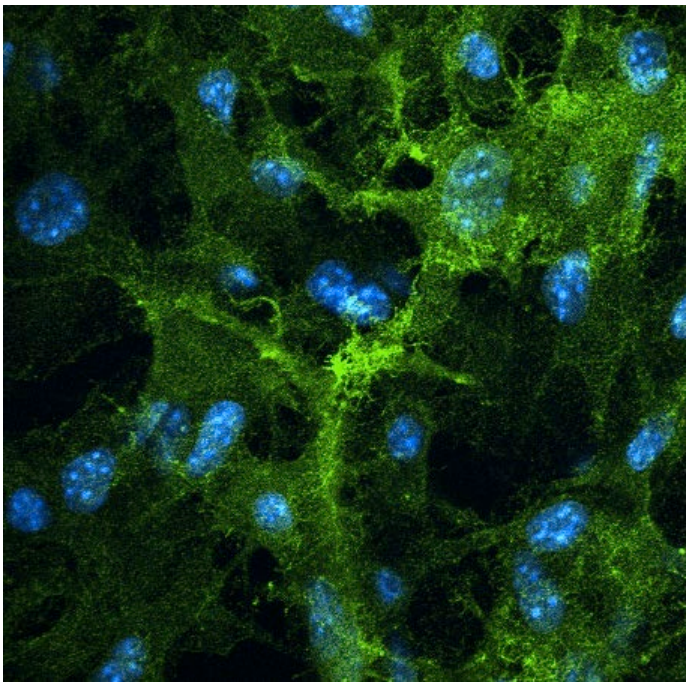
### ***Immunoprecipitation of mouse Podoplanin from CHO transfectant***

- Lane 1: IP with isotype control (M081-3, 1  $\mu\text{g}$ )
  - Lane 2: IP with D321-3, 0.5  $\mu\text{g}$
  - Lane 3: IP with D321-3, 1  $\mu\text{g}$
  - 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<sub>2</sub> 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<sup>®</sup>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)



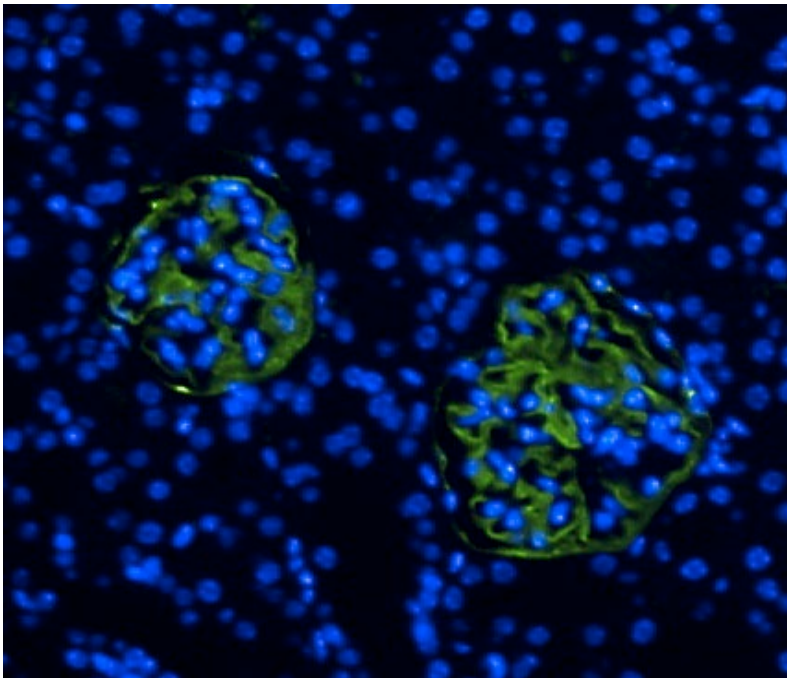
***Immunocytochemical detection of mouse Podoplanin in B16-F10***

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<sup>®</sup>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)



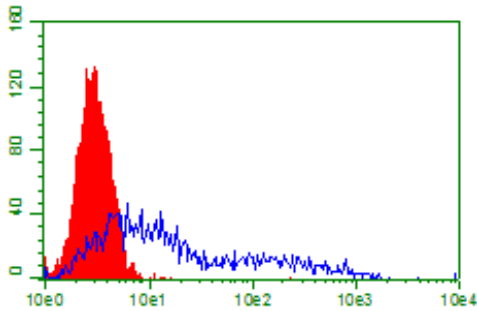
***Immunohistochemical detection of mouse Podoplanin in mouse kidney***

Green: D321-3  
Blue: DAPI

### **Flow cytometric analysis**

- 1) Wash the cells ( $5 \times 10^5$  cells/sample) 3 times with 1 mL of washing buffer (PBS containing 2% fetal calf serum (FCS)).
- 2) Add 100  $\mu$ 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  $\mu$ 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  $\mu$ 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  $\mu$ L of 1:400 anti-IgG (Rat)-Alexa Fluor<sup>®</sup>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  $\mu$ 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)