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

Anti-Podoplanin (Human) mAb

CODE No. D320-3

CLONALITY Monoclonal
CLONE NZ-1.2
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	0.1-1 µg/mL
Immunoprecipitation	0.5-1 µg/200 µL of cell extract from 1 x 10^6 cells
Immunohistochemistry	0.25-1 µg/mL
Flow cytometry	0.1 µg/mL

APPLICATIONS-UNDER EVALUATION

Immunocytochemistry

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Cells	Transfectant, H226, U87MG	Transfectant, Colon-26, B16-F10	Transfectant	Transfectant
Reactivity	+	–	–	–

Entrez Gene ID 10630 (Human)

REFERENCES

1) Kaji, C., *et al.*, *Acta. Histochem. Cytochem.* **45**, 227-237 (2012)
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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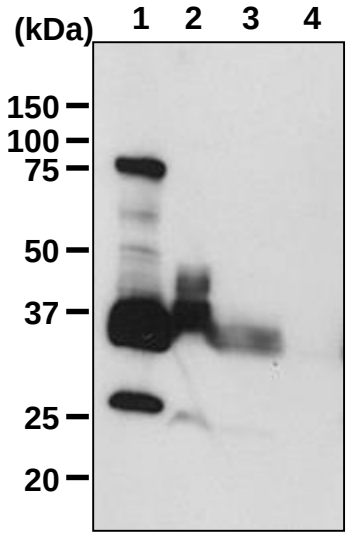
SDS-PAGE & Western blotting

1) Wash 1 x 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 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% methanol). 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).
8) Incubate the membrane with an HRP conjugated anti-rat IgG antibody 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).
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, H226, U87MG)

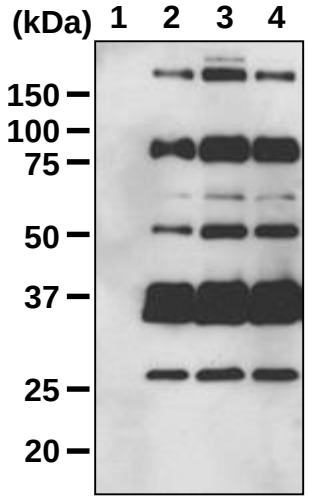


Western blot analysis of Podoplanin
Lane 1: human Podoplanin/CHO
Lane 2: H226
Lane 3: U87MG
Lane 4: H522 (negative control)
Immunoblotted with D320-3

Immunoprecipitation

1) Wash 5 x 10^6 cells twice 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) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with1 mL of IP buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) twice.
8) Add 200 μL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hr. at room temperature.
9) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
10) Resuspend the agarose with 1 mL of Lysis buffer.
11) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
12) Repeat steps 10)-11) 5 times.
13) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3 min. and centrifuge.
14) Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
15) 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% methanol). See the manufacturer's manual for precise transfer procedure.
16) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
17) 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.)
18) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3).
19) Incubate the membrane with an HRP conjugated anti-rat IgG antibody diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
20) Wash the membrane with PBS-T (5 min. x 3).
21) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
22) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
23) 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)



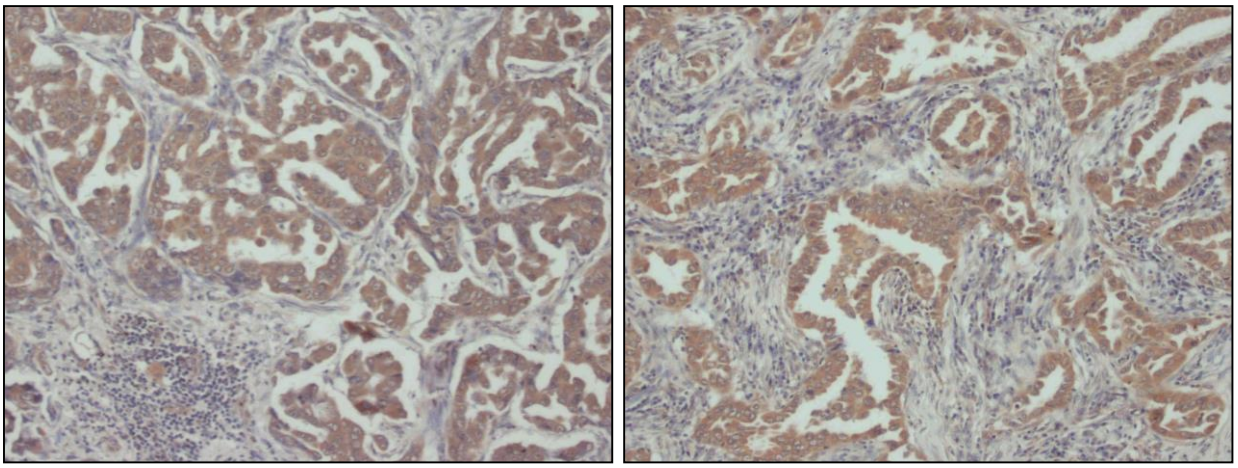
Immunoprecipitation of Podoplanin from transfectant

Lane 1: IP with isotype control (M081-3, 1 μg)
Lane 2: IP with D320-3, 0.5 μg
Lane 3: IP with D320-3, 1 μg
Lane 4: human Podoplanin/CHO, whole cell lysate
Immunoblotted with D320-3

Immunohistochemical staining for paraffin-embedded sections

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 the PBS and cover each section with 3% H_2O_2 in PBS for 10 min. at room temperature to block endogenous peroxidase activity.
5) Wash the slides with PBS 3 times for 5 min. each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with blocking buffer [20 mM HEPES (pH 7.2), 1% BSA, 135 mM NaCl] for 5 min. at room temperature to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with 1% BSA/PBS as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the slides 3 times in PBS for 5 min. each.
9) Wipe gently around each section and cover tissues with a biotin conjugated anti-rat IgG antibody. Incubate at room temperature.
10) Wash the slides 3 times in PBS for 5 min. each.
11) Wipe gently around each section and cover tissues with an HRP conjugated streptavidin. Incubate at room temperature.
12) Wash the slides 3 times in PBS for 5 min. each.
13) Visualize by reacting for 10 min. with DAB substrate solution (MBL, code no. 8469). *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
14) Wash the slides in water for 5 min.
15) Counter stain in hematoxylin for 1 min., wash the slides 3 times in water for 5 min. each, and then immerse the slides in PBS for 5 min. Dehydrate by immersing in Ethanol 3 times for 3 min. each, followed by immersing in Xylene 3 times for 3 min. each.
16) Now ready for mounting.

(Positive control for Immunohistochemistry; human lung cancer)

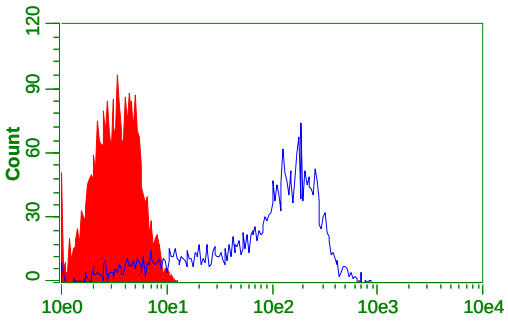


Immunohistochemical detection of Podoplanin in human lung cancer
Immunohistochemical staining with D320-3

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).
2) Add 100 µL of 4% paraformaldehyde/PBS to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
3) Wash the cells twice 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 30 min. at room temperature.
6) Wash the cells once with 1 mL of washing buffer.
7) Add 40 µL of 1:400 anti-IgG (Rat)-Alexa Fluor® 488 (Thermo Fisher Scientific, code no. A11006) diluted with the washing buffer. Mix well and incubate for 20 min. at room temperature.
8) Wash the cells once 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 Podoplanin in transfectant

Open: D320-3
Closed: isotype control (M081-3)