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

MONOCLONAL ANTIBODY

Anti-Human Podocalyxin/PCLP1

Code No.	Clone	Subclass	Quantity	Concentration
M085-3	4H11	Mouse IgG2a	100 µL	1 mg/mL

BACKGROUND: Recent studies with avian embryos and murine embryonic stem cells have suggested that hematopoietic cells are derived from hemangioblasts, the common precursors of hematopoietic and endothelial cells. Hara et al. molecularly cloned podocalyxin-like protein 1 (PCLP1) as a novel surface marker for endothelial-like cells in the AGM (aorta-gonad-mesonephros) region of mouse embryos, where long-term repopulating hematopoietic stem cells (LTR-HSCs) are known to arise. $PCLP1^{+}$ $CD45^{-}$ cells in the AGM region incorporated acetylated low-density lipoprotein and produced both hematopoietic and endothelial cells when cocultured with OP9 stromal cells. Moreover, multiple lineages of hematopoietic cells were generated in vivo when $PCLP1^{+}$ $CD45^{-}$ cells were injected into neonatal liver of busulfan-treated mice. Today it is reported that the PCLP1 is identical with the Podocalyxin.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose beads. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c splenocyte immunized with CHO cell expressing full length human Podocalyxin/PCLP1.

FORMULATION: 100 µg IgG in 100 µL volume of 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.

REACTIVITY: This antibody reacts with human Podocalyxin/PCLP1.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow Cytometry; 10-20 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Podocalyxin /PCLP1 transfected CHO cell	Not Tested	Not Tested
Reactivity on WB	+		

INTENDED USE:

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REFERENCES:

1) Schopperle, WM., *et al., Biochem. Biophys. Res. Commun*. **300**, 285-290 (2003)
2) Doyonnas, R., *et al., J. Exp. Med*. **194**, 13-27 (2001)
3) Hara, T., *et al., Immunity* **11**, 567-78 (1999)
4) Kershaw, DB., *et al., J. Biol. Chem.* **272**, 15708-15714 (1997)

RELATED PRODUCTS:

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)
M084-3 Anti-Human Podocalyxin/PCLP1 (53D11)
M084-4 FITC Labeled Anti-Human Podocalyxin/PCLP1 (53D11)

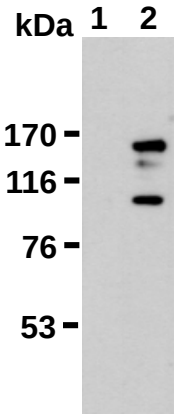
PROTOCOLS:

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-polyacrylamide gel for electrophoresis.

5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour 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 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 ^{o}C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody to be used will be depend on condition.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 POD-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS (5 minutes x 6 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 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; transfectant)



Western blot analysis of Podocalyxin/ PCLP1 protein
Lane 1: Parental Cell (CHO)
Lane 2: Transfectant (hPodocalyxin/PCLP1-CHO)
Immunobltted with M085-3

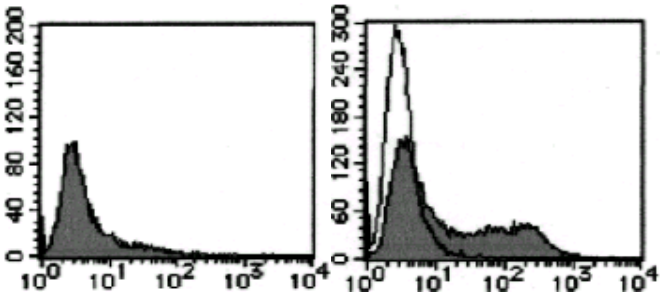
Flow cytometric analysis for floating cells

Protocol 1

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
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^{o}C). Remove supernatant by careful aspiration.
4) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) and 0.1% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 30 µL of the Anti-Human Podocalyxin/PCLP1 monoclonal antibody (4H11) (10-20 µg/mL) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) 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.
7) Add 30 µL of secondary antibody (1:40 FITC conjugated anti-mouse IgG/MBL code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) 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.
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 analysis of human Podocalyxin/PCLP1 expression on transfectant
Left: Parental cell (CHO)
Right: Transfectant (hPodocalyxin/PCLP1-CHO)
■ M085-3
□ Isotype contrl