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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-Podocalyxin (PCLP1) (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M084-3	53D11	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 co-cultured 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; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow Cytometry; 10-20 µg/mL (final concentration)

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

INTENDED USE:
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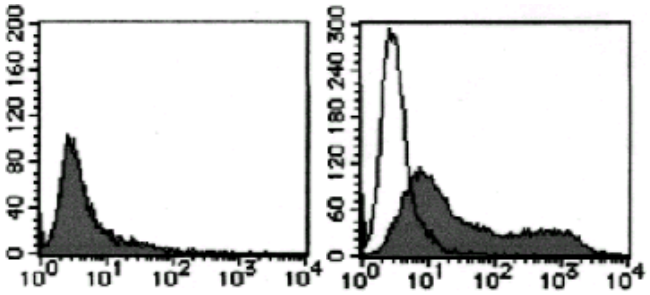
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Podocalyxin /PCLP1 transfected CHO cell	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:
1) Schopperle, W. M., *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**, 567-578 (1999)
4) Kershaw, DB., *et al., J. Biol. Chem.* **272**, 15708-15714 (1997)



Flow cytometric analysis of human Podocalyxin/PCLP1 expression on transfectant
Left: Parental cell (CHO)
Right: Transfectant (hPodocalyxin/PCLP1-CHO)
■ M084-3
□ Isotype contrl

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

PROTOCOL:

Flow cytometric analysis for floating cells

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 0.09% NaN_3].
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
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°C). Remove supernatant by careful aspiration.
4) Add 10 μL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) and 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 30 μL of the primary antibody diluted with the washing buffer as suggested in the **APPLICATIONS**. 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 the secondary antibody (FITC conjugated anti-mouse IgG antibody) 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)

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