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

MONOCLONAL ANTIBODY

Anti-Podocalyxin (PCLP1) (Human) mAb-FITC

Code No.	Clone	Subclass	Quantity	Concentration
M084-4	53D11	Mouse IgG2a	100 µL	500 µg/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 (53D11) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with CHO cell expressing full-length human Podocalyxin (PCLP1).

FORMULATION: 50 µg IgG in 100 µL volume of PBS containing 1% BSA 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.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with human Podocalyxin (PCLP1) on Flow cytometry.

APPLICATION:
Flow cytometry; 30 µg/mL (final concentration)
*Please refer to the data sheet (MBL code no. M084-3) for other applications.

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

INTENDED USE:
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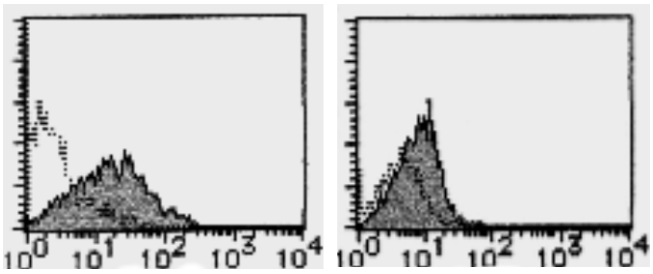
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	HUVEC, MCF7	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:
1) Kuhara, M., *et al.*, *Cell Struct. Funct.* **34**, 23-30 (2009) [FCM]
2) Doyonnas, R., *et al.*, *Blood* **105**, 4170-4178 (2005)
3) Minegishi, N., *et al.*, *Blood* **102**, 896-905 (2003)
4) Schopperle, M.W., *et al.*, *Biochem. Biophys. Res. Commun.* **300**, 285-290 (2003)
5) Minehata, K., *et al.*, *Blood* **99**, 2360-2368 (2002)
6) Doyonnas, R., *et al.*, *J. Exp. Med.* **194**, 13-27 (2001)
7) Hara, T., *et al.*, *Immunity* **11**, 567-578 (1999)
8) Kershaw, B.D., *et al.*, *J. Biol. Chem.* **272**, 15708-15714 (1997)

This antibody is used in reference number 1).

RELATED PRODUCTS:
D072-3 Anti-Podocalyxin (PCLP1) (Mouse) mAb (10B9)
D072-4 Anti-Podocalyxin (PCLP1) (Mouse) mAb-FITC (10B9)
D072-5 Anti-Podocalyxin (PCLP1) (Mouse) mAb-PE (10B9)
D072-6 Anti-Podocalyxin (PCLP1) (Mouse) mAb-Biotin (10B9)
M084-3 Anti-Podocalyxin (PCLP1) (Human) mAb (53D11)
M084-5 Anti-Podocalyxin (PCLP1) (Human) mAb-PE (53D11)
M085-3 Anti-Podocalyxin (PCLP1) (Human) mAb (4H11)



Flow cytometric analysis of Podocalyxin/ PCLP1 expression on HUVEC cells (left) and MCF7 cells (right). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of M084-4 to the cells.*

PROTOCOL:
Flow cytometric analysis for adherent cells
We usually use Fisher tubes or equivalents as reaction tubes for all steps after 2).
1) Detach the cells from culture dish by using cell

dissociation buffer (Invitrogen; code no. 13151-014).

2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
3) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
4) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
5) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
6) Add 20 µL of the primary antibody as suggested in the **APPLICATIONS**. Mix well and incubate for 30 minutes at room temperature.
7) 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.
8) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; HUVEC and MCF7)