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

MONOCLONAL ANTIBODY

Anti-Mouse Podocalyxin/PCLP1-Biotin

Code No.	Clone	Subclass	Quantity	Concentration
D072-6	10B9	Rat IgG1	1 mL	50 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. Anti-Mouse PCLP1 stains LO cells as well as the endothelial-like cells in the AGM culture. LO cell is a novel OSM (oncostatin M)-dependent cell line which exhibits characteristics similar to endothelial-like cells. Today it is reported that the PCLP1 is identical with the Podocalyxin.

SOURCE: This antibody was purified from mouse ascites fluid using protein G agarose. This hybridoma (clone 10B9) was established by fusion of mouse myeloma cell P3X with Wister rat lymph node immunized with LO cells.

FORMULATION: 50 μg IgG in 1 mL 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 mouse Podocalyxin/PCLP1 on Flow cytometry.

APPLICATIONS:
Flow cytometry; 10-20 μg/mL (final concentration)
*Please refer to the data sheet (MBL; code no. D072-3) for other applications.

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not Tested	LO	Not Tested
Reactivity on FCM		+	

INTENDED USE:
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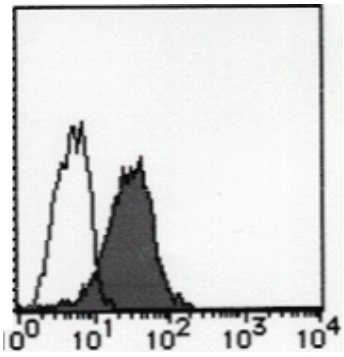
REFERENCES:
1) Onitsuka, I., *et al.*, *Gastroenterology*, **138**, 1525-1535 (2010)
2) Doyonnas, R., *et al.*, *Blood* **105**, 4170-4178 (2005)
3) Minegishi, N., *et al.*, *Blood* **102**, 896-905 (2003)
4) Minehata, K., *et al.*, *Blood* **99**, 2360-2368 (2002)
5) Doyonnas, R., *et al.*, *J. Exp. Med.* **194**, 13-28 (2001)
6) Hara, T., *et al.*, *Immunity* **11**, 567-578 (1999)

Clone 10B9 is used in these references.

RELATED PRODUCTS:

D072-3	anti-Mouse Podocalyxin/PCLP1 (10B9)
D072-4	anti-Mouse Podocalyxin/PCLP1-FITC (10B9)
D072-5	anti-Mouse Podocalyxin/PCLP1-PE (10B9)
M084-3	anti-Human Podocalyxin/PCLP1 (53D11)
M084-4	anti-Human Podocalyxin/PCLP1-FITC (53D11)
M085-3	anti-Human Podocalyxin/PCLP1 (4H11)
M080-3	Rat IgG1 isotype control (1H5)
M080-4	Rat IgG1 isotype control-FITC (1H5)
M080-5	Rat IgG1 isotype control-PE (1H5)



Flow cytometric analysis of mouse Podocalyxin/PCLP1 expression on LO cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D072-6 to the cells.*

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PROTOCOL:

Flow cytometric analysis for adherent cells

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

1) Wash the cells 2 times with PBS.
2) Incubate LO cells in Cell Dissociation Buffer (Invitrogen; cat. no. 13151-014) for 30 minutes at 37^{o}C.
3) Harvest and resuspend the cells with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
4) Harvest and count the cells (2 x 10^5 cells/tube).
5) Centrifuge at 500 x g for 1 minute at room temperature (20~25^{o}C). Remove supernatant by careful aspiration.
6) Add 10 μL of normal goat serum to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
7) Add 40 μL of the primary antibody at the concentration of as suggest in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 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) Add 30 μL of 1:40 diluted FITC conjugated streptavidin with the washing buffer (MBL; code no. IM-0307) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) 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.
11) (Optional) Add 20 μL of PE Labeled anti-mouse CD45 (MBL; code no. IM-2817) and mix gently. Incubate for 15 minutes at room temperature.
12) (Optional) 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.
13) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LO)