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

MONOCLONAL ANTIBODY

Anti-Mouse Podocalyxin/PCLP1

Code No.	Clone	Subclass	Quantity	Concentration
D072-3	10B9	Rat IgG1	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. 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 lymphnode immunized with LO cells.

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 mouse Podocalyxin/PCLP1 on Flow cytometry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 5-10 µg/mL (final concentration)

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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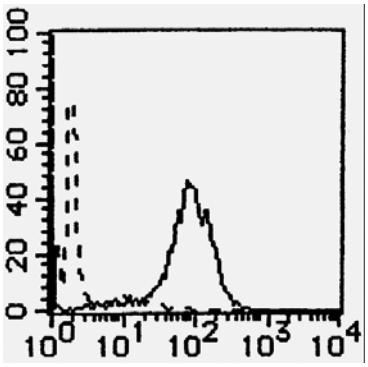
REFERENCES:

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:

D072-4	anti-Mouse Podocalyxin/PCLP1-FITC (10B9)
D072-5	anti-Mouse Podocalyxin/PCLP1-PE (10B9)
D072-6	anti-Mouse Podocalyxin/PCLP1-Biotin (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. *Dotted line indicates the reaction of isotypic control to the cells. Solid line indicates the reaction of D072-3 to the cells.*

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PROTOCOL:

Flow cytometric analysis for LO cell

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 as suggested 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 secondary antibody 1:40 FITC conjugated anti-rat IgG (MBL; code no. IM-0827) 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) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LO)