

MONOCLONAL ANTIBODY

Anti-Mouse Podocalyxin/PCLP1-FITC

| Code No. | Clone | Subclass | Quantity | Concentration |
|----------|-------|----------|----------|---------------|
| D072-4 | 10B9 | Rat IgG1 | 1 mL | 20 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 lymphnode immunized with LO cells.

FORMULATION: 20 µg IgG in 1 mL volume of PBS containing 1% BSA and 0.09% NaN₃.
*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.

APPLICATION:

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:

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

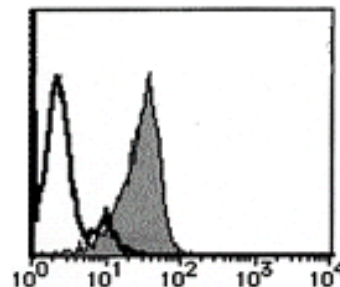
REFERENCES:

- 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)
- D072-5 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. Open histogram indicates the reaction of Isotypic control to the cells. Shaded histogram indicates the reaction of D072-4 to the cells.

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°C.
- 3) Harvest and resuspend the cells with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN₃].
- 4) Harvest and count the cells (2 x 10⁵ cells/tube).
- 5) Centrifuge at 500 x g for 1 minute at room temperature (20~25°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 20 µL of the FITC labeled anti-Mouse Podocalyxin /PCLP1 monoclonal antibody (10B9). Mix well and incubate for 30 minutes at room temperature.
(Optional) Add 20 µL of PE labeled anti-mouse CD45 (MBL; code no. IM-2817) in parallel and mix gently.
- 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; LO)