

Product datasheet for **AM26622AF-N**

PODXL Mouse Monoclonal Antibody [Clone ID: 53D11]

Product data:

Product Type:	Primary Antibodies
Clone Name:	53D11
Applications:	FC, IHC
Recommended Dilution:	Flow cytometry: 10-20 µg/ml (final concentration). For details see protocol below. Immunohistochemistry: Clone 53D11 has been reported by customer to stain Podocalyxin in Formalin-Fixed, Paraffin-Embedded human kidney tissue at an antibody concentration of 10 µg/ml.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	CHO cell expressing full length human Podocalyxin/PCLP1
Specificity:	This antibody reacts with human Podocalyxin/PCLP1.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	podocalyxin like
Database Link:	Entrez Gene 5420 Human O00592



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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.

Synonyms: Podocalyxin-like protein 1, PCLP, PCLP1, PCLP-1, GCTM-2 antigen, Hematopoietic Stem Cell Marker

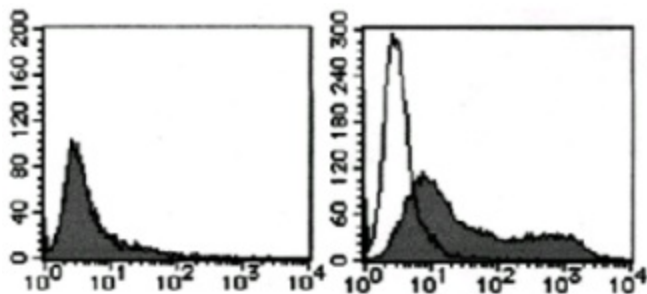
Note: This product was originally produced by MBL International.

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 0.1% NaN₃].
- 2) Resuspend the cells with washing buffer (5x10⁶ 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) and 0.1% NaN₃ to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 5) Add 30 µL of the Anti-Human Podocalyxin/PCLP1 monoclonal antibody (53D11) (10-20 µg/mL) diluted with the washing buffer. 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 30 µL of secondary antibody (1:40 FITC conjugated anti-mouse IgG) 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)

Product images:



Flow cytometric analysis of human Podocalyxin/PCLP1 expression on transfectant
 Left: Parental cell (CHO)
 Right: Transfectant (hPodocalyxin/PCLP1-CHO)
 Black: AM26622AF-N
 White: Isotype control