Datasheet for ABIN5541392

anti-PODXL antibody

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Overview

Quantity:	0.1 mL
Target:	PODXL
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This PODXL antibody is un-conjugated
Application:	Western Blotting (WB), Flow Cytometry (FACS)

Product Details

Immunogen:	CHO cell expressing full length human Podocalyxin/PCLP1
Clone:	4H11
Isotype:	IgG2a
Specificity:	This antibody reacts with human Podocalyxin/PCLP1.
Purification:	Protein A agarose beads

Target Details

Target:	PODXL
Alternative Name:	podocalyxin,podxl (PODXL Products)
Background:	Recent studies with avian embryos and murine embryonic stem cells have suggested that hematopoietic cells are derived from hemangioblasts, the common precursors of

Target Details

	hematopoietic and endothelial cells. Hara et al. molecularly clone d 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 lipopro tein 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.
UniProt:	O00592
Pathways:	Tube Formation

Application Details

Application Notes:	Western blot: 1 µg/mL for chemiluminescence detection system. Flow cytometry: 10 - 20 µ g/mL (final concentration). For details see protocosl below.
Protocol:	SDS-PAGE & Western Blotting 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1 % NP-40, 2 mM EDTA, 10 % glycerol) containing appropriate protease inhibitors. Incubate it at 4 o C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds). 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 o C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution. 3) Mix the sample with equal volume of Laemmli's sample buffer. 4) Boil the samples for 2 minutes and centrifuge. Load 10 µ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis. 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm 2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20 % MeOH). See the manufacture's manual for precise transfer procedure. 6) To reduce nonspecific binding, soak the membrane in 10 % skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 o C. 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1 % skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody to be used will be depend on condition.) 8) Wash the membrane with PBS (5 minutes x 6 times). 9) Incubate the membrane with the 1:10,000 POD-conjugated anti-mouse IgG diluted with 1 % skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. 10) Wash the membrane with PBS (5 minutes x 6 times). 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemilumin escence reagent for 1 minute. Remove extra reagent from the

Application Details

	membrane by dabbing with paper towel, and seal it in plastic wrap. 12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary. (Positive control for Western blotting transfectant) Flow cytometric analysis for floating cells Protocol 1 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 % NaN 3]. 2) Resuspend the cells with washing buffer (5x10e6 cells/mL). 3) Add 50 µ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25 o C). Remove supernatant by careful aspiration. 4) Add 10 µ L of Clear Back (human Fc receptor blocking reagent) and 0.1 % NaN 3 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 (4H11) (10-20 µ g/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 µ L of the washing buffer and analyze by a flow cytometer. (Positive control for flow cytometry transfectant)
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	PBS containing 50 % glycerol, pH 7.2. No preservative is contained.
Preservative:	Azide free
Storage:	-20 °C
Storage Comment:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: One year from despatch.