Mouse Podocalyxin Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1556

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects mouse Podocalyxin in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 1% cross-reactivity with recombinant human (rh) Podocalyxin and rhEndoglycan is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse Podocalyxin Ser21-Arg402 Accession # Q9R0M4
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

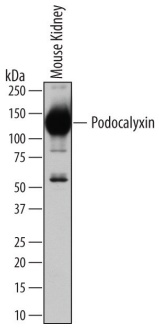
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Flow Cytometry	0.25 µg/10^6 cells	D3 mouse embryonic stem cell line; Neuro-2A mouse neuroblastoma cell line
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

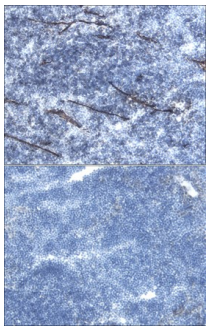
DATA

Western Blot



Detection of Mouse Podocalyxin by Western Blot. Western blot shows lysates of mouse kidney tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Mouse Podocalyxin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1556) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for Podocalyxin at approximately 130 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



Podocalyxin in Mouse Thymus. Podocalyxin was detected in perfusion fixed frozen sections of mouse thymus using Goat Anti-Mouse Podocalyxin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1556) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling when primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. Specific staining was localized to high endothelial venules. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

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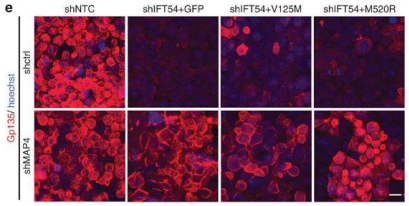
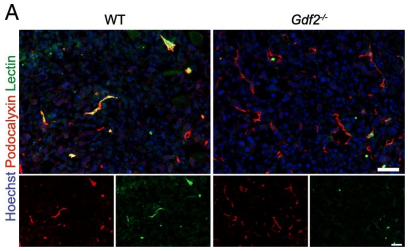
Immunocytochemistry/ Immunofluorescence



Detection of Mouse Podocalyxin Like by Immunocytochemistry/Immunofluorescence Defects in TRAF3IP1 mutants are mediated by MAP4.(a) Lateral views of WT zebrafish embryos injected with map4 morpholino at 48 hpf and phenotype distribution in WT embryos injected with control or map4 morpholino. (b) Lateral views of elipsa zebrafish embryos injected with map4 morpholino at 48 hpf and phenotype distribution in elipsa mutant embryos injected with control or map4 morpholino (data shown as combined result of n=3 independent experiments). Scale bars, 1 mm. (c) Relative expression of Map4 normalized to that of Hprt was analysed by qPCR in control and Traf3ip1-KD mIMCD3 cells stably expressing GFP or GFP-IFT54 mutants and Map4 shRNA. (d) Control and Traf3ip1-KD/ Map4-KD mIMCD3 cells expressing either GFP or IFT54-GFP fusions were fixed in MeOH and stained for acetylated α-tubulin (red) and γ-tubulin (light blue). Scale bar, 10 µm. (e) Six hours after Ca2+ switch, mIMCD3 cells grown until confluence on filters were fixed with 4% PFA and stained for the apical marker Gp135 (red). Scale bar, 10 µm. (f) Percentage of normal spheroids of control and Traf3ip1-KD/ Map4-KD mIMCD3 cells expressing either GFP or IFT54-GFP fusions grown on Matrigel for 5 days (mean ± s.d., n≥100 spheroids from 3 independent experiments, ***P≤0,0001, *P<0.012, Bonferonni's multiple-comparison test). Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms9666), licensed under a CC-BY license. Not internally tested by R&D Systems.

Immunocytochemistry/ Immunofluorescence



Detection of Mouse Podocalyxin Like by Immunocytochemistry/Immunofluorescence Gdf2 deletion decreases tumor perfusion and maturation in the E0771 mammary cancer model. E0771 cells were injected in the 4th mammary gland of WT and Gdf2−/− mice and tumor vascularization was analyzed 9 days after tumor detection. a Representative images of the tumors stained for podocalyxin (red), lectin (green) and cell nuclei (blue, Hoechst). Scale bar 50 µm. b Vascular density quantified by podocalyxin positive area (% of tumor area) and (c) assessment of vessel diameter using Ferret's theorem (WT n = 7, Gdf2−/−n = 13, 1 representative experiment out of 2). d Quantification of vessel perfusion by lectin staining (% area of lectin/podocalyxin) (WT n = 8, Gdf2−/− n = 7, 1 representative experiment out of 3). e Representative images of the tumors stained for podocalyxin (red), α-smooth muscle actin (α-SMA) (green) and cell nuclei (blue, Hoechst). Scale bar 100 µm. f α-SMA staining quantification (% area of α-SMA/podocalyxin) (WT n = 8, Gdf2−/− n = 7, 1 representative experiment out of 3). b, c, d, f Data are the median ± interquartile range. Statistical analysis: Mann-Whitney test. *p ≤ 0.05 and **p ≤ 0.01 significantly different Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30165893), licensed under a CC-BY license. Not internally tested by R&D Systems.

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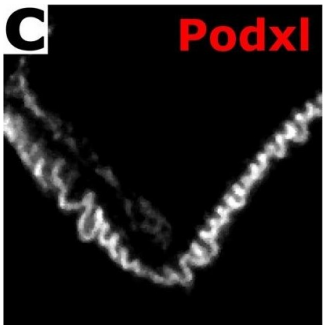
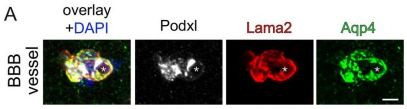


Detection of Mouse Podocalyxin Like by Immunocytochemistry/Immunofluorescence Confocal ExM images of mouse kidney labeled with antibodies or fluorescent proteins. (a–c) Single focal plane of glomerulus immunostained for podocin (a), agrin (b), podocalyxin (Podxl c), and merge (d) of (a–c). (e–g) Confocal maximum intensity projections of glomerulus immunostained for synaptopodin (Synpo, e), acetylated tubulin (acTub, f), podocin (g) highlighting secondary FPs, primary FPs, and slit diaphragms/FP boundaries, respectively. (h) Merge of (e–h). (i–k) Confocal maximum intensity projections of glomerulus immunostained for collagen IV (Coll IV, i), podocalyxin (Podxl, j), and α smooth muscle actin (αSMA, k) and highlighting Bowman's capsule and the mesangium, podocytes, and arterioles and the mesangium, respectively. (l) Merge of (i–k). (m–p) Single focal plane of glomerulus showing native fluorescence from confetti mouse expressing YFP (m) and RFP (o) in separate podocyte cell bodies and FPs as well as GFP (n) in various podocyte nuclei. (p) Merge of (m–o). (q) Zoomed-in view of region highlighted in (p). (r) Further zoomed-in view (top) and cross-sectional profile (bottom) of boxed region highlighted in (q). All distances and scale bars are in pre-expansion units. Scale bars, 2 µm (a–h,q), 25 µm (i–l), 5 µm (m–p). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29991751), licensed under a CC-BY license. Not internally tested by R&D Systems.

Immunocytochemistry/ Immunofluorescence



Detection of Mouse Podocalyxin Like by Immunocytochemistry/Immunofluorescence Endothelial β-catenin GOF does not affect the ECM of astrocytic endfeet and ECs within the subfornical organ (SFO).Striatal BBB-vessel showing a polarized distribution of Lama2 and Aqp4 in AC endfeet. Lumen is stained by Podxl (asterisk) (A). Coronal overview of the subfornical organ (SFO) (B); rectangular inset demarcates area for higher magnification in (C). Striatal BBB-vessel showing a polarized distribution of CollV (green) but no Meca32 (white) in ECs (D). Coronal overview SFO, rectangular inset demarcates area for higher magnification in F (E); white dashed lines show Meca32+, red dashed lines show Meca32 vessels (F). Dashed lines outline SFO vessels; scale bars show 2 µm (A), 50 µm (B), 10 µm (C), 2.5 µm (D), 50 µm (E), 10 µm (F). Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30932814), licensed under a CC-BY license. Not internally tested by R&D Systems.

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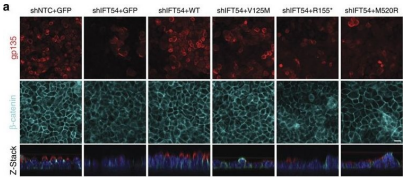
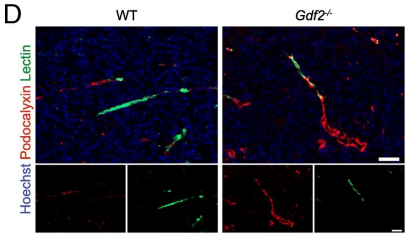


Detection of Mouse Podocalyxin Like by Immunocytochemistry/Immunofluorescence TRAF3IP1 mutations lead to epithelialization and polarity defects.(a) mIMCD3 cells grown until confluence on filters were subjected to Ca2+-free medium to disrupt the tight junctions. Six hours after Ca2+ addition, cells were analysed by immunofluorescence using the apical marker Gp135 (red) and β-catenin (light blue) to stain the cell junctions. Scale bar, 10 µm. (b) Following Ca2+ switch, tight junction re-formation was assessed by measurement of trans-epithelial resistance (TER) at different time points (mean ± s.e.m. of n=5 independent experiments, two-way ANOVA; NS: not-significant, ***P<0.001 at 6 h). (c) Height of mIMCD3 cells grown on filters measured as the distance from the base to the top of the cells (GFP staining, not shown; mean ± s.d. of n≥20, from 3 independent experiments, ***P<0.001, Bonferonni's multiple-comparison test). (d) Expression of the apical marker Gp135 was analysed by Western blot with α-tubulin as a loading control. (e) mIMCD3 cells grown in matrigel 3D matrix to form spheroids were stained for ZO1 (tight junctions, red) and analysed by confocal microscopy. Arrows indicate ZO-1 at the apical junctions, while arrow heads point to mislocalized ZO-1. Equatorial sections of representative spheres are shown for each cell line. Scale bars, 10 µm. (f) Percentage of abnormal spheroids (no/small lumen filled with cells) (mean ± s.d., n=80 spheroids from 2 independent experiments, ***P≤0,001, **P<0.002, Bonferonni's multiple-comparison test). Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/ncomms9666), licensed under a CC-BY license. Not internally tested by R&D Systems.

Immunocytochemistry/ Immunofluorescence



Detection of Mouse Podocalyxin Like by Immunocytochemistry/Immunofluorescence Bmp10 conditional deletion has no impact on tumor growth, angiogenesis and lung metastasis in the E0771 mammary cancer model. a Schematic representation of the experimental protocol for Bmp10 specific deletion and E0771 cells implantation. Tamoxifen was injected in all 3-week-old mice; 3 weeks later, E0771 cells were injected and tumor growth was analyzed for 3 weeks. b Plasmatic levels of BMP10 in control (CTL, n = 15) and Bmp10 conditional KO (Bmp10-cKO, n = 15) mice assessed by ELISA at the end of the experiment. c Tumor growth was assessed by caliper measurement every 2 to 3 days after tumor detection (CTL n = 7, Bmp10-cKO n = 8, 1 representative experiment out of 3). d Representative images of the tumors stained for podocalyxin (red), lectin (green) and cell nuclei (blue, Hoechst). Scale bar 50 µm. e Vascular density quantified by podocalyxin surface area (% of tumor area) and (f) Quantification of vessel perfusion by lectin staining (% area of lectin/podocalyxin) (CTL n = 7, Bmp10-cKO n = 8, 1 representative experiment out of 3). g Total area, (h) number and (i) mean size of lung metastases per mice bearing metastases (CTL n = 10, Bmp10-cKO n = 9, 2 experiments). c Data are the mean ± SEM. Statistical analysis: Two-way matched ANOVA. b, e, f, g, h, i Data are the median ± interquartile range. Statistical analysis: Mann-Whitney test. ****p ≤ 0.001 significantly different Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30165893), licensed under a CC-BY license. Not internally tested by R&D Systems.

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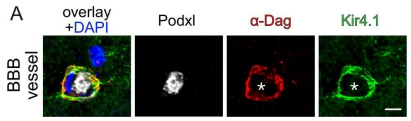

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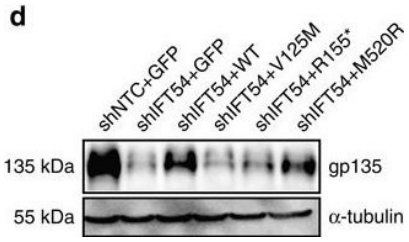
Western Blot



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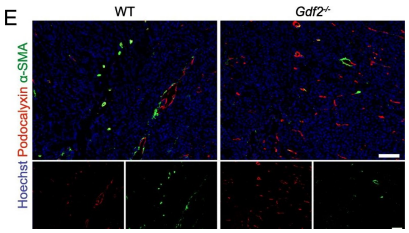

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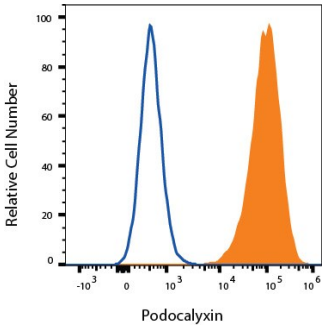
Flow Cytometry



Detection of Podocalyxin in Neuro-2A cells by Flow Cytometry Neuro-2A cells were stained with Goat Anti-Mouse Podocalyxin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1556, filled histogram) or isotype control antibody (Catalog # 4-001-A, open histogram) followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). View our protocol for Staining Membrane-associated Proteins.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Podocalyxin, also known as Podocalyxin-like protein-1 (PCLP1 or PODXL), is a type I transmembrane glycoprotein. It belongs to the CD34/Podocalyxin family of sialomucins that share structural similarity and sequence homology. Podocalyxin is a major sialoprotein in the podocytes of the kidney glomerulus and is also expressed by both endothelium and multipotent hematopoietic progenitors. It has been identified as a novel cell surface marker for hemangioblasts, the common precursors of hematopoietic and endothelial cells (1, 2).

References:

1. Li, J. *et al.* (2001) DNA Seq. **12**(5):407.
2. Hara, T. *et al.* (1999) Immunity **11**(5):567.

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