

Product Datasheet

Podocalyxin Like Antibody - BSA Free NB110-41503

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB110-41503

Podocalyxin Like Antibody - BSA Free

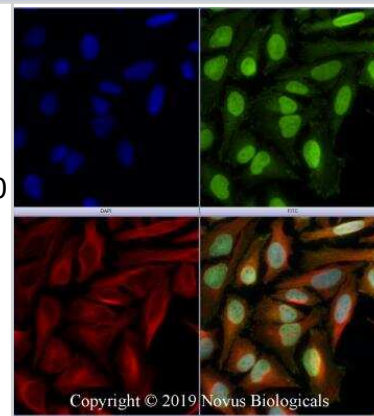
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	5420
Gene Symbol	PODXL
Species	Human, Mouse
Marker	Podocytes /Apical Marker, Endothelial Cell Marker
Immunogen	A synthetic peptide within an internal region (residues 100-200) of the human Podocalyxin protein. [Swiss-Prot: O00592] This immunogen should be far enough away from any glycosylation site.

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 2 ug/ml, Immunocytochemistry/ Immunofluorescence 1:1000
Application Notes	In Western blot, a band is observed at ~52 kDa. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors. In ICC/IF membrane staining was observed.

Images

Immunocytochemistry/Immunofluorescence: Podocalyxin Like Antibody [NB110-41503] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-Podocalyxin Like Antibody at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Publications

Lavecchia AM, Mantzouratou P, Cerullo D et al. Thyroid hormone treatment counteracts cellular phenotypical remodeling in diabetic organs iScience 2023-10-20 [PMID: 37752946]

Benedetti V, Brizi V, Guida P et al. Engineered kidney Tubules for Modeling Patient-Specific Diseases and Drug Discovery. EBioMedicine. 2018-07-01 [PMID: 30049385] (ICC/IF, Human)



Procedures

Western Blot Protocol specific for PODXL Antibody (NB110-41503)

Podocalyxin Like Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH₂O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-Podolyxin primary antibody (NB 110-41503) in blocking buffer and incubate 2 hours at room temperature.
8. Rinse the membrane in dH₂O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (Pierce, ECL).

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry/Immunofluorescence Protocol for Podolyxin antibody (NB110-41503)

Podocalyxin Like Antibody:

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.



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Products Related to NB110-41503

NB110-41503PEP	Podocalyxin Like Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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