

Product Datasheet

Nephrin Antibody - BSA Free NBP3-18058

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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NBP3-18058

Nephrin Antibody - BSA Free

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

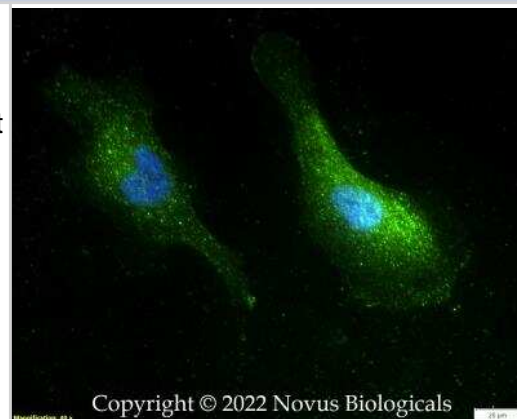
Product Description	
Description	Novus Biologicals Rabbit Nephrin Antibody - BSA Free (NBP3-18058) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	4868
Gene Symbol	NPHS1
Species	Human, Mouse
Immunogen	Partial recombinant protein made to the internal portion of human Nephrin (amino acids 23-1029) [UniProt O60500].

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Immunohistochemistry 1:200, Immunocytochemistry/Immunofluorescence 1 - 2 ug/ml, Immunohistochemistry-Paraffin 1:200

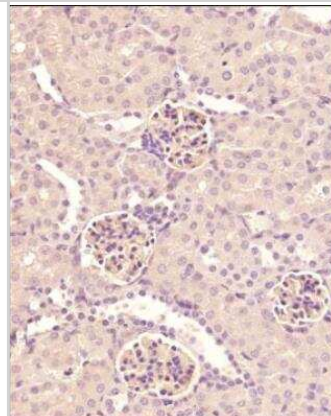


Images

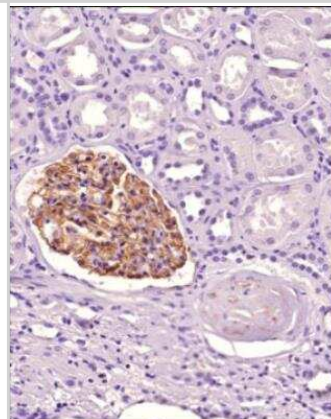
Immunocytochemistry/Immunofluorescence: Nephrin Antibody [NBP3-18058] - RCC4 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with Nephrin Antibody (NBP3-18058) at 1ug/ml overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunohistochemistry-Paraffin: Nephrin Antibody [NBP3-18058] - Analysis of a FFPE tissue section of mouse kidney using 1:200 dilution of Nephrin antibody (NBP3-18058). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



Immunohistochemistry-Paraffin: Nephrin Antibody [NBP3-18058] - Analysis of a FFPE tissue section of human kidney using 1:200 dilution of Nephrin antibody (NBP3-18058). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



Procedures

Immunocytochemistry/Immunofluorescence Protocol for Nephrin Antibody (NBP3-18058)

Immunocytochemistry Protocol

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

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.

Immunohistochemistry-Paraffin Protocol for Nephrin Antibody (NBP3-18058)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:

Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.



Western Blot Protocol for Nephrin Antibody (NBP3-18058)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 1% Non-fat milk, 1% BSA in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three 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.





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Products Related to NBP3-18058

NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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