

# Product Datasheet

## Podocin/NPHS2 Antibody - BSA Free NBP3-18129

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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[technical@novusbio.com](mailto:technical@novusbio.com)

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**NBP3-18129**

Podocin/NPHS2 Antibody - BSA Free

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

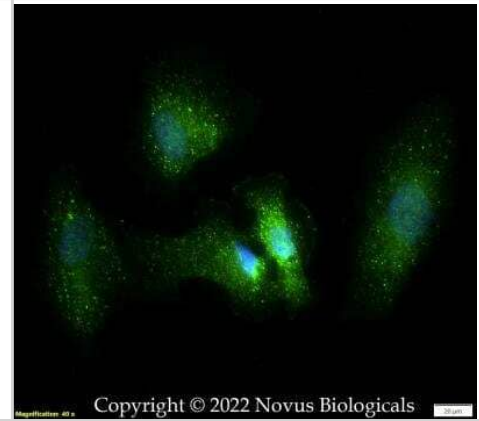
<b>Product Description</b>	
<b>Host</b>	Rabbit
<b>Gene ID</b>	7827
<b>Gene Symbol</b>	NPHS2
<b>Species</b>	Human, Mouse
<b>Immunogen</b>	Partial recombinant protein made to an internal portion of human Podocin/NPHS2 (between amino acids 250-400) [UniProt Q9NP85].

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

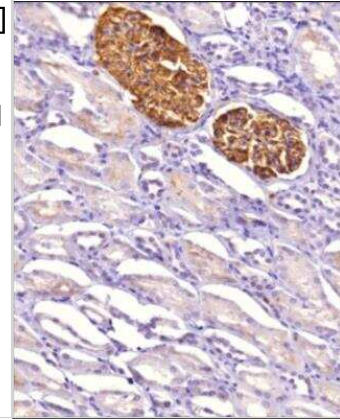


## Images

Immunocytochemistry/Immunofluorescence: Podocin/NPHS2 Antibody [NBP3-18129] - 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 Podocin/NPHS2 Antibody (NBP3-18129) 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: Podocin/NPHS2 Antibody [NBP3-18129] - Analysis of a FFPE tissue section of human kidney using 1:200 dilution of Podocin/NPHS2 antibody (NBP3-18129). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.



## Publications

Liu C, Cheng Q, Ao Q et al. Induced pluripotent stem cells-podocytes promote repair in acute kidney injury is dependent on Mafk/CCR5/Nampt axis-mediated M2 macrophage polarization *Chemico-biological interactions* 2023-08-01 [PMID: 37182688]

## Procedures

### Western Blot Protocol for Podocin/NPHS2 Antibody (NBP3-18129)

#### 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 5% Non-fat milk 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.

### Immunohistochemistry-Paraffin Protocol for Podocin/NPHS2 Antibody (NBP3-18129)

#### 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.



**Immunocytochemistry/Immunofluorescence Protocol for Podocin/NPHS2 Antibody (NBP3-18129)**

## 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.





### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NBP3-18129**

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HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control
NBP2-33570PEP	Podocin/NPHS2 Recombinant Protein Antigen

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### **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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