

# Product Datasheet

## NHE3/SLC9A3 Antibody - BSA Free NBP3-00589

Unit Size: 0.1 mg

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

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### Publications: 1

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**NBP3-00589**

NHE3/SLC9A3 Antibody - BSA Free

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

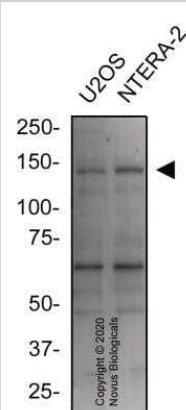
Product Description	
Description	Novus Biologicals Rabbit NHE3/SLC9A3 Antibody - BSA Free (NBP3-00589) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. Anti-NHE3/SLC9A3 Antibody: Cited in 1 publication. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	6550
Gene Symbol	SLC9A3
Species	Human
Immunogen	Partial synthetic peptide made to an internal portion of the human NHE3 protein (between amino acids 600-660) [UniProt P48764]

Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 2 ug/ml, Immunohistochemistry 1:200, Immunocytochemistry/Immunofluorescence 5 - 10 ug/ml, Immunohistochemistry-Paraffin 1:200



## Images

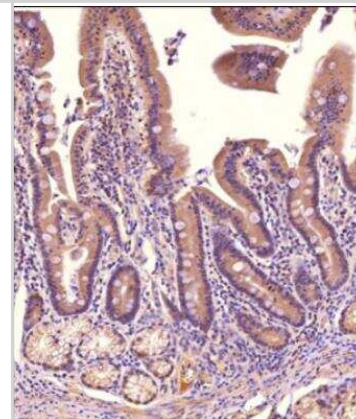
**Western Blot: NHE3/SLC9A3 Antibody [NBP3-00589]** - Total protein from human Hek293 and HeLa cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-NHE3 (NBP3-00589) in blocking buffer and detected with an anti-rabbit HRP secondary antibody using NovaLume chemiluminescence detection reagent (NPB2-61915).



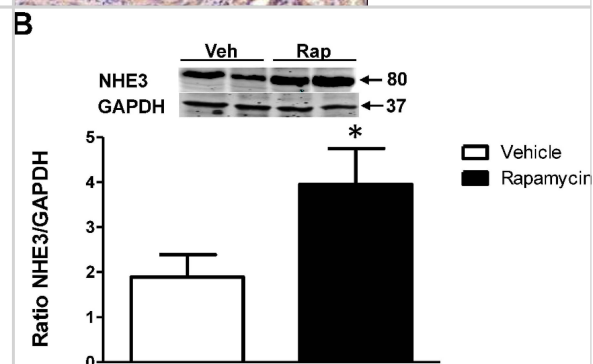
**Immunocytochemistry/Immunofluorescence: NHE3/SLC9A3 Antibody [NBP3-00589]** - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-NHE3/SLC9A3 at 5 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



**Immunohistochemistry-Paraffin: NHE3/SLC9A3 Antibody [NBP3-00589]** - Analysis of a FFPE tissue section of human small intestine using 1:200 dilution of NHE3 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin. This NHE3 antibody generated nuclear staining.



**Rapamycin treatment increases DNMT1 and NHE3 expression in hRPTCs.** Cells were treated with rapamycin at the concentrations of 1, 10, 20, and 50 nM or vehicle for 24 h. The expression of DNMT1 was determined by Western blot. Quantification was normalized by tubulin expression. The molecular size markers are indicated on the left. Another set of hRPTCs were treated for 24 h with 50 nM rapamycin or vehicle, and the expression of NHE3 of the cell lysates was determined by Western blot. Quantification was normalized by GAPDH. The molecular size markers are indicated on the left. n = 3/group \* p < 0.05 vs. vehicle. Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/36430934>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Armando I, Cuevas S, Fan C et al. G Protein-Coupled Receptor 37L1 Modulates Epigenetic Changes in Human Renal Proximal Tubule Cells International journal of molecular sciences 2022-11-21 [PMID: 36430934] (WB, Human)

## Procedures

### Western Blot Protocol for NHE3/SLC9A3 Antibody (NBP3-00589)

#### 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 manufacture

### Immunocytochemistry/Immunofluorescence Protocol for NHE3/SLC9A3 Antibody (NBP3-00589)

#### 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. Permeablize 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 NHE3/SLC9A3 Antibody (NBP3-00589)**

## 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.





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### **Products Related to NBP3-00589**

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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]
NBP3-00589PEP	NHE3/SLC9A3 Antibody Blocking Peptide

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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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