Product Datasheet

WNK1 Antibody
NB600-225

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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Publications: 1

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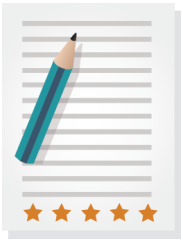


NB600-225

WNK1 Antibody

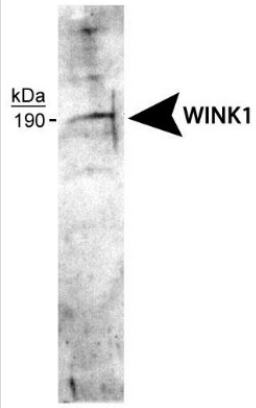
Product Information	
Unit Size	0.1 ml
Concentration	5 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	190 kDa

Product Description	
Host	Rabbit
Gene ID	65125
Gene Symbol	WNK1
Species	Human, Mouse, Rat
Reactivity Notes	Human, mouse and rat.
Immunogen	A synthetic peptide within the C-terminal region of human WNK1 (within the last 350 amino acids). [UniProt# Q9H4A3]

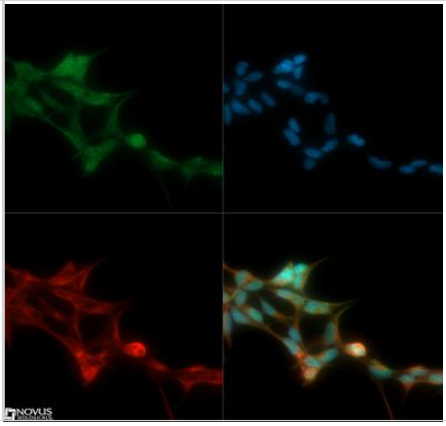
Product Application Details	
Applications	Western Blot, Immunocytochemistry/Immunofluorescence
Recommended Dilutions	Western Blot 1:1000, Immunocytochemistry/Immunofluorescence 1:500 - 1:1000
Application Notes	This WNK1 antibody is useful for Immunocytochemistry/Immunofluorescence and Western blot. In WB, this antibody recognizes the short form of WNK1, ~190 kDa. The theoretical molecular weight of human WNK1 is ~251 kDa and rat WNK1 is ~230 kDa. In ICC/IF cytomplasmic staining was observed. 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.

Images

Western Blot: WNK1 Antibody [NB600-225] - Detection of the short form of WNK1 in F-11 cell lysate.



Immunocytochemistry/Immunofluorescence: WNK1 Antibody [NB600-225] - WNK1 antibody was tested in HEK293 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Publications

Wade, JB et al. WNK1 kinase isoform switch regulates renal potassium excretion. Proc Natl Acad Sci U S A;103 (22):8558-63. 2006 May 30. [PMID: 16709664]

Procedures

Western Blot Protocol for WNK1 antibody (NB600-225)
Western Blot Protocol
1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 25 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% NFDM + 1% BSA in TBS + Tween, 1 hour at RT.
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-WNK1 primary antibody (NB600-225) in blocking buffer and incubate 1 hour 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 WNK1 antibody (NB600-225)
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 1:25,0000 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 NB600-225

NB600-225PEP	WNK1 Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP (Horseradish Peroxidase)]
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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