

Product Datasheet

UCH-L1/PGP9.5 Antibody - BSA Free NB300-675SS

Unit Size: 0.025 ml

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

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NB300-675SS

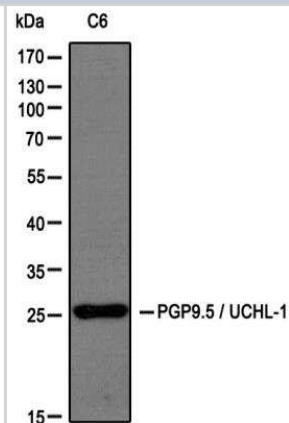
UCH-L1/PGP9.5 Antibody - BSA Free

Product Information	
Unit Size	0.025 ml
Concentration	1 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
Target Molecular Weight	25 kDa
Product Description	
Description	Novus Biologicals Rabbit UCH-L1/PGP9.5 Antibody - BSA Free (NB300-675) is a polyclonal antibody validated for use in IHC, WB and ICC/IF. Anti-UCH-L1/PGP9.5 Antibody: Cited in 3 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	7345
Gene Symbol	UCHL1
Species	Human, Mouse, Guinea Pig
Reactivity Notes	Guinea Pig reactivity reported in scientific literature (PMID: 31739508).
Marker	pan-Neuronal Marker
Immunogen	A synthetic peptide made to an internal portion of the human UCHL1 protein (between residues 100-200). [UniProt# P09936]
Product Application Details	
Applications	Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry/Immunofluorescence, Immunohistochemistry
Recommended Dilutions	Western Blot 0.5-2.0 ug/ml, Immunohistochemistry 2.5-5.0 ug/ml, Immunocytochemistry/ Immunofluorescence 1:100-1:200, Immunohistochemistry-Paraffin 2.5-5.0 ug/ml
Application Notes	This UCHL1 antibody is useful for Immunocytochemistry/Immunofluorescence, Western blot and Immunohistochemistry-Paraffin. By Western blot a band at ~25 kDa is seen.

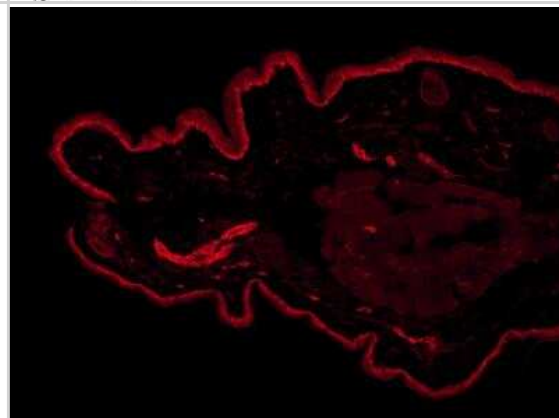


Images

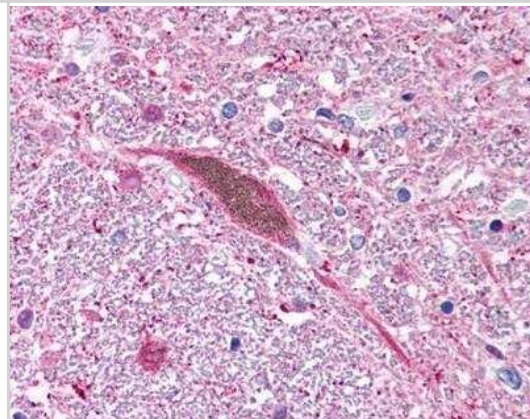
Western Blot: UCH-L1/PGP9.5 Antibody [NB300-675] - Analysis of extracts from C6 cells using PGP9.5 / UCHL-1 antibody (NB300-675, 1:200). Image from verified customer review.



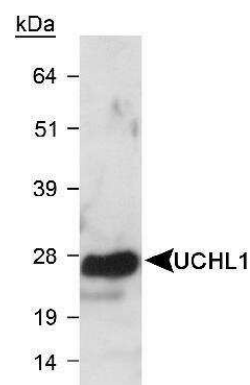
Immunocytochemistry/Immunofluorescence: UCH-L1/PGP9.5 Antibody [NB300-675] - Analysis of acetone-fixed, frozen mouse ear skin section using anti-PGP9.5 antibody. Image from verified customer review.



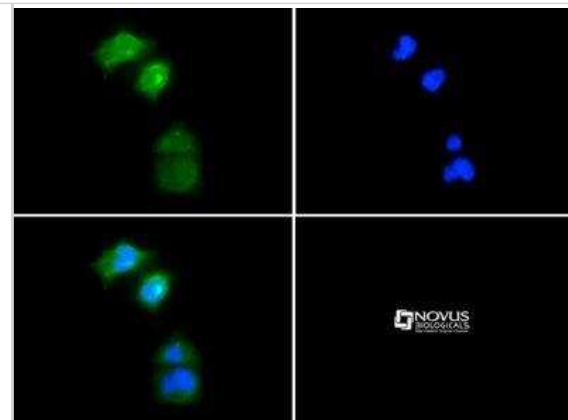
Immunohistochemistry: PGP9.5 / UCHL-1 Antibody [NB300-675] - Human brain, Substantia Nigra, Pigmented Neuron, 40X.



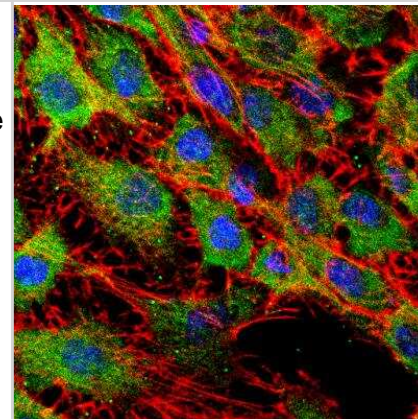
Western Blot: PGP9.5 / UCHL-1 Antibody [NB300-675] - Detection of UCHL1 in mouse brain lysate.



Immunocytochemistry/Immunofluorescence: UCH-L1/PGP9.5 Antibody [NB300-675] - In Neuro2a cells with FITC (green). Nuclei were counterstained with DAPI (blue).



Immunocytochemistry/Immunofluorescence: UCH-L1/PGP9.5 Antibody [NB300-675] - Confocal analysis of C6 cells using PGP9.5 / UCHL-1 antibody (NB300-675, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue). Image from verified customer review.



Publications

Saraswathula A, Liu MM, Kulaga H, Lane AP Chronic interleukin-13 expression in mouse olfactory mucosa results in regional aneuronal epithelium International forum of allergy & rhinology 2022-08-11 [PMID: 35950767] (Mouse)

Chiocchetti R, Hitrec T, Giancola F et al. Phosphorylated Tau protein in the myenteric plexus of the ileum and colon of normothermic rats and during synthetic torpor Cell 2021-01-29 [PMID: 33511469]

Saver AE, Crawford SA, Joyce JD, Bertke AS Route of Infection Influences Zika Virus Shedding in a Guinea Pig Model Cells 2019-11-14 [PMID: 31739508] (IF/IHC, Guinea Pig)

Procedures

Protocol specific for UCHL1 Antibody (NB300-675)

UCH-L1/PGP9.5 Antibody:

Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 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 for 2 hours 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-UCHL1 primary antibody (NB 300-675) 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 manufacturer's 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 manufacturer's instructions (Pierce's 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.

IHC-FFPE sections

I. Deparaffinization:

- A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
- B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:

- A. Place slides in peroxidase quenching solution: 15-30 minutes. To Prepare 200 ml of Quenching Solution: Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
- B. Place slides in distilled water: 2 changes for 2 minutes each.



III. Retrieve Epitopes:

- A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celsius.
- B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
- C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
- D. Slowly add distilled water to further cool for 5 minutes.
- E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:

- A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap-Pen).
- B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
- C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
- D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
- E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
- G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
- H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
- I. Wash slides with Wash Solution: 3 changes for 5 minutes each. Wash slides with Wash Solution: 3 changes for 5 minutes each
- J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
- K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
- L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
- M. Rinse slides in distilled water.
- N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
- O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
- P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
- Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.

-Prior to deparaffinization, heat slides overnight in a 60 degrees Celsius oven.

-All steps in which Xylene is used should be performed in a fume hood.

-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.

-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.

-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.

-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.

-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1.5 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).





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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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