Product Datasheet

UCH-L1/PGP9.5 Antibody - BSA Free
NB300-676

Unit Size: 0.1 ml

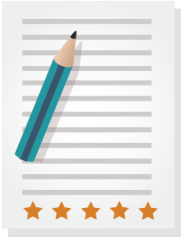
Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Reviews: 1 Publications: 3

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB300-676

Updated 4/13/2025 v.20.1



NB300-676

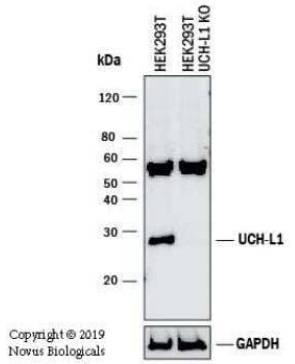
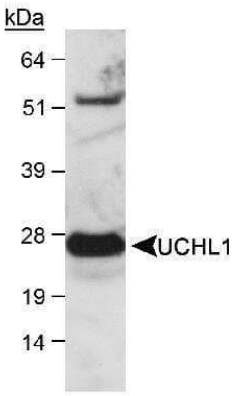
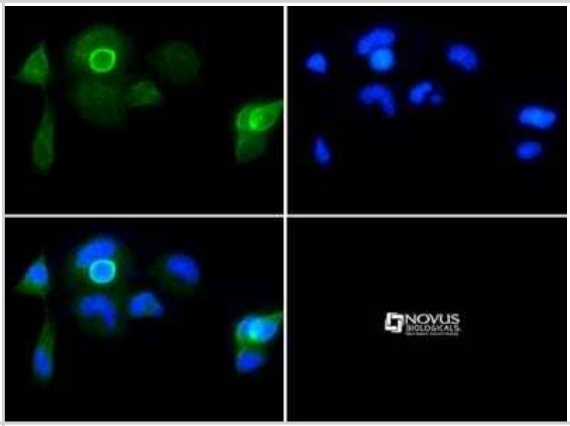
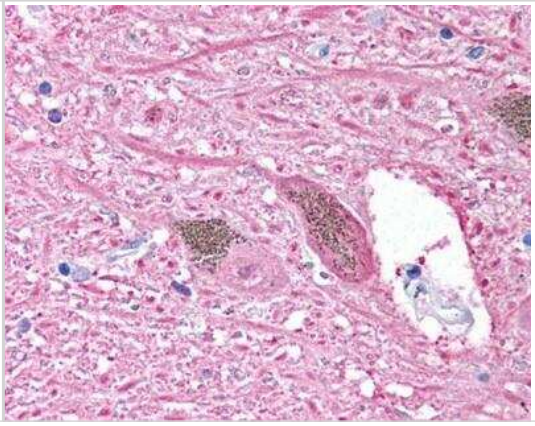
UCH-L1/PGP9.5 Antibody - BSA Free

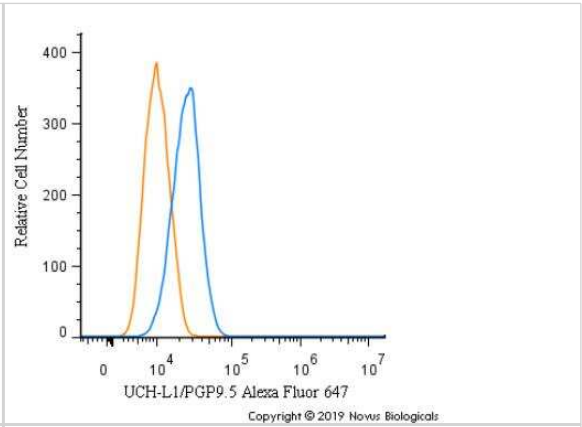
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Glycine, 0.15 M NaCl
Target Molecular Weight	25 kDa

Product Description	
Host	Rabbit
Gene ID	7345
Gene Symbol	UCHL1
Species	Human, Mouse
Marker	pan-Neuronal Marker
Immunogen	A synthetic peptide made to a C-terminal portion of the human UCHL1 protein (between residues 200-220). [UniProt# P09936]

Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Knockout Validated
Recommended Dilutions	Western Blot 2 ug/mL, Simple Western 1:2000, Flow Cytometry 2-5 ug/million cells/ml, Immunohistochemistry 2.5 - 5.0 ug/mL, Immunocytochemistry/ Immunofluorescence 1:100 - 1:200, Immunohistochemistry-Paraffin 2.5 - 5.0 ug/mL, Immunohistochemistry-Frozen reported in scientific literature (PMID 25041530), Knockout Validated
Application Notes	By Western blot a band at ~25 kDa is seen. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Human Brain lysate 0.05 mg/mL, separated by Size, antibody dilution of 1:2000, apparent MW was 29 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. 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

Knockout Validated: UCH-L1/PGP9.5 Antibody [NB300-676] - Western blot shows lysates of HEK293T human embryonic kidney parental cell line and UCH-L1/PGP9.5 knockout (KO) HEK293T cell line. PVDF membrane was probed with 2 ug/ml of Rabbit Anti-Human UCH-L1/PGP9.5 Polyclonal Antibody (Catalog # NB300-676) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog #HAF008). Specific band was detected for UCH-L1/PGP9.5 at approximately 28 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in the knockout HEK293T cell line. This experiment was conducted under reducing conditions.	
Western Blot: UCH-L1/PGP9.5 Antibody [NB300-676] - Analysis of UCHL1 in mouse brain lysate ECL detection, 60 seconds.	
Immunocytochemistry/Immunofluorescence: UCH-L1/PGP9.5 Antibody [NB300-676] - Neuro2a cells stained with FITC (green). Nuclei were counterstained with DAPI (blue).	
Immunohistochemistry-Paraffin: UCH-L1/PGP9.5 Antibody [NB300-676] - Staining of neurons and cell processes at 2.5ug/mL. Human brain, Substantia Nigra, Pigmented Neurons, 4X.	

Flow Cytometry: UCH-L1/PGP9.5 Antibody [NB300-676] - An intracellular stain was performed on U-87 cells with UCH-L1/PGP9.5 Antibody NB300-676AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.	
Simple Western: UCH-L1/PGP9.5 Antibody [NB300-676] - Image shows a specific band for PGP9.5/UCHL-1 in 0.05 mg/mL of Human Brain lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	

Publications

Awad-Igbaria Y, Dadon S, Shamir A et al. Characterization of Early Inflammatory Events Leading to Provoked Vulvodynia Development in Rats Journal of inflammation research 2022-07-11 [PMID: 35845089]

Powis RA, Mutsaers CA, Wishart TM et al. Increased levels of UCHL1 are a compensatory response to disrupted ubiquitin homeostasis in spinal muscular atrophy and do not represent a viable therapeutic target. Neuropathol. Appl. Neurobiol. 2014-07-08 [PMID: 25041530] (IHC-Fr, WB, Mouse, Human)

Details:
UCHL-1 antibody used for WB (1:2000 dilution) and IHC-Fr (1:500 dilution) - see full text for detailed methods. WB performed on Type I SMA patient primary fibroblasts (Figure 1), 'Taiwanese' SMA mouse spinal cord and skeletal (Figure 2) and E17 rat hippocampal neurons treated or not with 50uM UBEI-41, a cell-permeable ubiquitin E1 inhibitor muscle (Figure 7). IHC was done on frozen sections of ventral horn of the spinal cord and gastrocnemius muscle of 'Taiwanese' SMA mouse (Figure 2 C and F).

Wang R, Zhang M, Zhou W et al. NF-kappaB signaling inhibits ubiquitin carboxyl-terminal hydrolase L1 gene expression. J Neurochem 2011-03-01 [PMID: 21104237] (WB, Human)

Procedures

Protocol specific for UCHL1 Antibody (NB300-676)
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 300-676) 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).

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 NB300-676

NB820-59657	Mouse Brain Whole Tissue Lysate (Adult Whole Normal)
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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB300-676

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications