

Product Datasheet

Histone H3 Antibody - BSA Free NB500-171

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB500-171

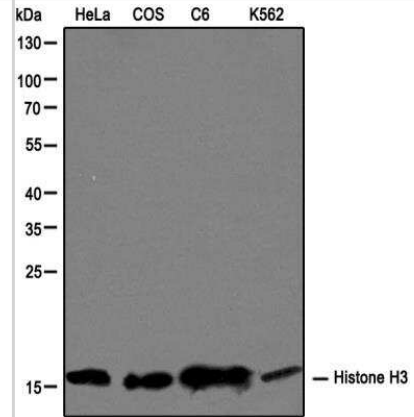
Histone H3 Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.01% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS (pH 7.4)
Target Molecular Weight	15 kDa
Product Description	
Host	Rabbit
Gene ID	126961
Gene Symbol	H3C14
Species	Human, Mouse, Rat, Insect, Xenopus, Yeast
Reactivity Notes	Predicted to react with many species based on 100% sequence homology including <i>C. elegans</i> , chicken, drosophila, and plant. Xenopus reactivity reported in scientific literature (PMID: 24048589). Insect (<i>Aedes aegypti</i>) reactivity reported from a verified customer review.
Immunogen	This Histone H3 antibody was raised against a synthetic peptide made to an C-terminal portion of the human Histone H3 protein (between residues 100-136) [UniProt P68431]
Product Application Details	
Applications	Western Blot, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation (ChIP), Single Cell Western
Recommended Dilutions	Western Blot 1:1000-1:4000, Immunohistochemistry 1:100-1:300, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 24048589), Immunohistochemistry-Paraffin 1:100-1:300, Immunoblotting reported in scientific literature (PMID 24048589), Chromatin Immunoprecipitation (ChIP), Single Cell Western
Application Notes	In Western Blot, a band is seen ~15 kDa. In IHC-P, nuclear staining was observed in human, mouse, and rat tissues. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

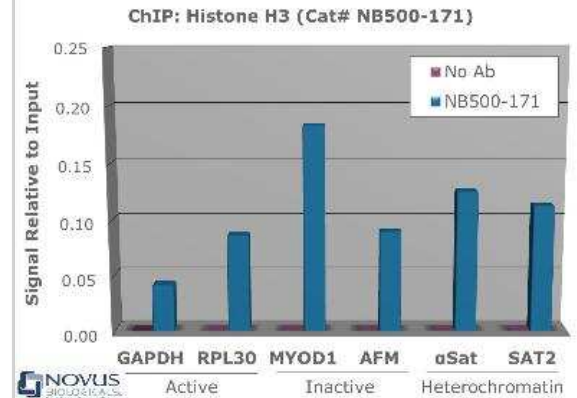


Images

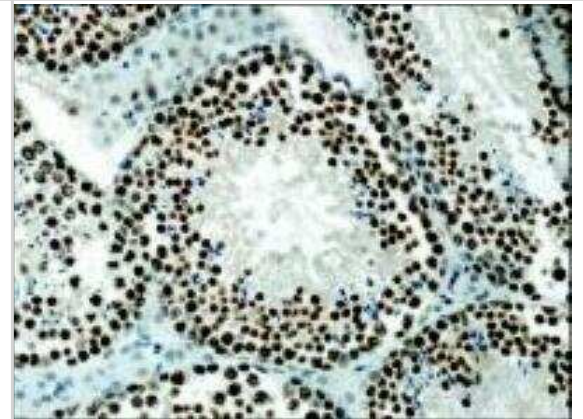
Western Blot: Histone H3 Antibody [NB500-171] - Analysis of HeLa, COS, C6, and K562 cell lysate using histone H3 antibody [NB500-171] antibody at 1:100. Observed molecular weight is ~17 kDa.



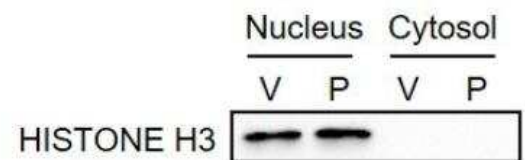
Chromatin Immunoprecipitation: Histone H3 Antibody [NB500-171] - Chromatin from one million formaldehyde cross-linked HeLa cells was precipitated using 2 ug of NB500-171 and 25 uL of magnetic IgG beads, using standard ChIP methods. A similar sample containing no antibody (No Ab) was included as a negative control. Immunoprecipitated DNA was quantified using quantitative real-time PCR and SYBR green dye, then normalized to the non-precipitated input chromatin. Representative target genes from active, inactive, and heterochromatic regions of the genome show amplification, indicative of the presence of Histone H3.



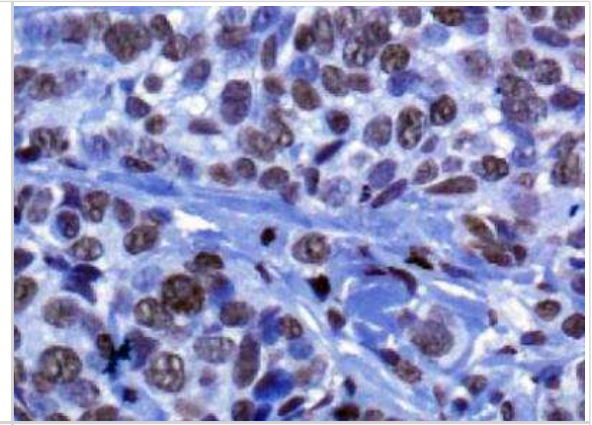
Immunohistochemistry-Paraffin: Histone H3 Antibody [NB500-171] - Immunoperoxidase staining of Anti-Histone H3 in FFPE mouse testis.



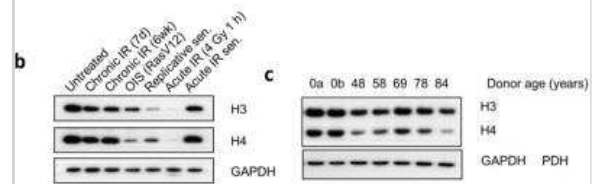
Western Blot: Histone H3 Antibody [NB500-171] - MDA-MB-231 cells were treated with vehicle (V) or paclitaxel (P). Cytosolic and nuclear lysates were prepared, and immunoblot assay was performed. WB image submitted by a verified customer review.



Immunohistochemistry: Histone H3 Antibody [NB500-171] - Analysis of Histone H3 in human breast cancer using DAB with hematoxylin counterstain.



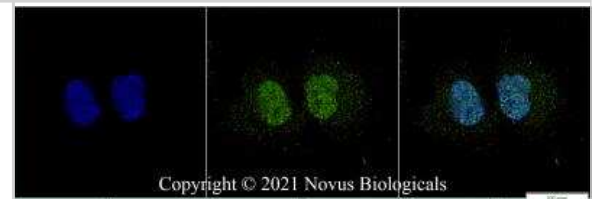
Western Blot: Histone H3 Antibody [NB500-171] - Reduced histone levels in senescent cells is induced in vitro by different means, and in vivo from aged donors. (b) Western immunoblot analyses of histones in fibroblasts described in (a). (c) Histone levels in dermal fibroblasts isolated from human neonatal (age 0, donors a and b) and adult donors. Anti- used at 1:20,000 and Anti-Histone H4 [ac Lys12, ac Lys16, ac Lys8, ac Lys5] (NBP2-16848) used at 1:10,000. Image collected and cropped by CiteAb from the following publication (<https://doi.org/10.1038/s41598-020-59163-4>) licensed under a CC-BY license.



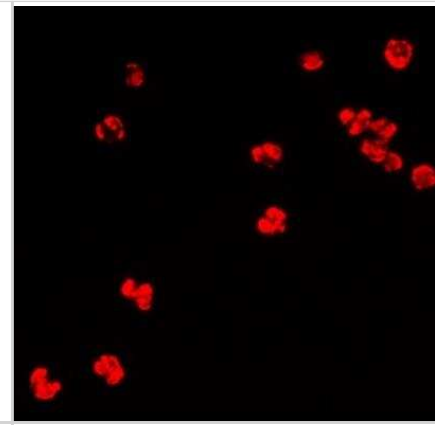
Immunocytochemistry/Immunofluorescence: Histone H3 Antibody [NB500-171] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NB500-171 at 1 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



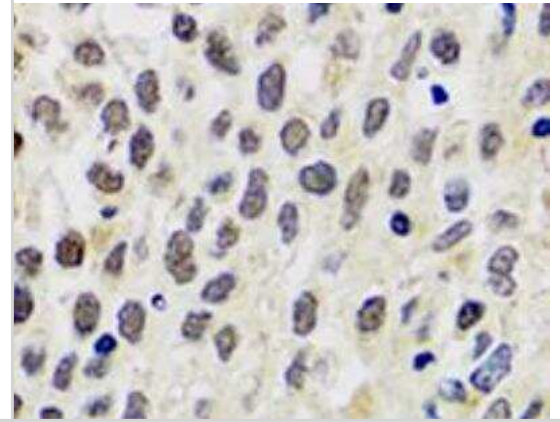
Immunocytochemistry/Immunofluorescence: Histone H3 Antibody [NB500-171] - PC12 cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti- NB500-171 at 1 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



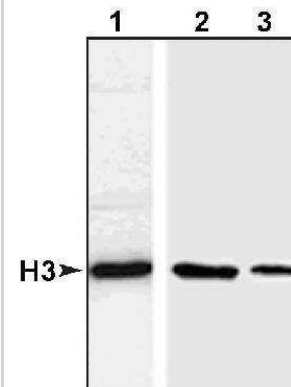
Immunocytochemistry/Immunofluorescence: Histone H3 Antibody [NB500-171] - Immunofluorescence staining to detect histone H3 in human blood. ICC/IF image submitted by a verified customer review.



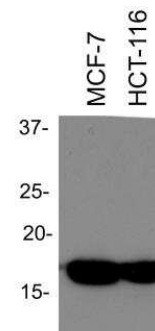
Immunohistochemistry-Paraffin: Histone H3 Antibody [NB500-171] - Analysis of Histone H3 antibody on Mouse pancreas tissue. Histone H3 antibody dilution 1:200. Image from verified customer review.



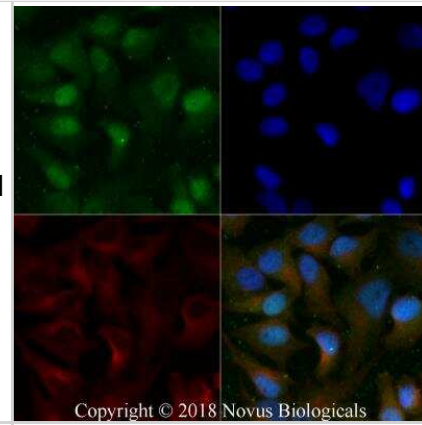
Western Blot: Histone H3 Antibody [NB500-171] - (1) HeLa, (2) *S. cerevisiae* whole cell lysates, (3) Histones purified from HeLa cells. Theoretical molecular weight is ~15 kDa.



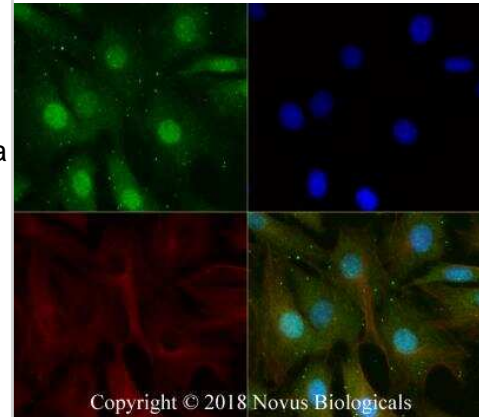
Western Blot: Histone H3 Antibody [NB500-171] - Total histone H3 levels in MCF-7 and HCT-116 cells. Observed molecular weight is ~17 kDa. WB image submitted by a verified customer review.



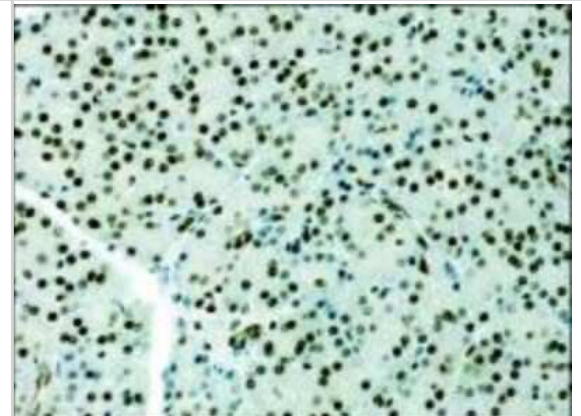
Immunocytochemistry/Immunofluorescence: Histone H3 Antibody [NB500-171] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-Histone H3 at 5 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



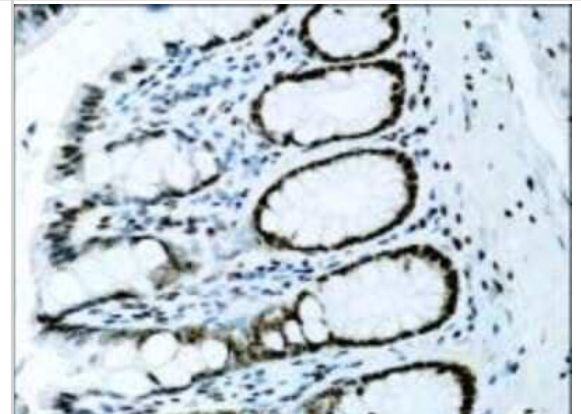
Immunocytochemistry/Immunofluorescence: Histone H3 Antibody [NB500-171] - NIH3T3 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton X-100. The cells were incubated with anti-Histone H3 at 5 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



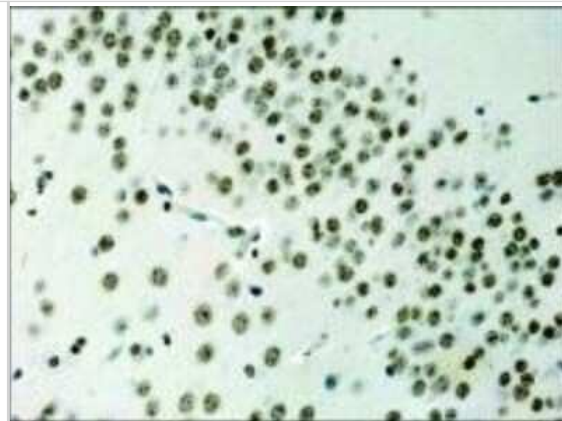
Immunohistochemistry-Paraffin: Histone H3 Antibody [NB500-171] - Immunoperoxidase staining of Anti-Histone H3 in FFPE rat pancreas.



Immunohistochemistry-Paraffin: Histone H3 Antibody [NB500-171] - Immunoperoxidase staining of Anti-Histone H3 in FFPE rat colon.



Immunohistochemistry-Paraffin: Histone H3 Antibody [NB500-171] - Immunoperoxidase staining of Anti-Histone H3 in FFPE mouse brain.

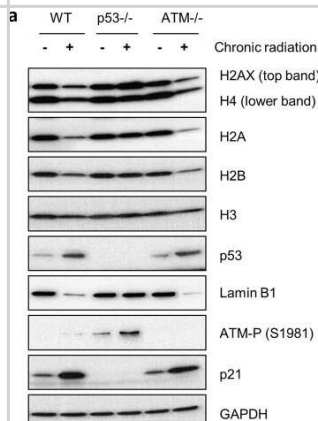


Histone H3 was detected in immersion fixed MCF7 human breast cancer cell line using Rabbit anti-Histone H3 Affinity Purified Polyclonal Antibody conjugated to FITC (Catalog # NB500-171F) (green) at 10 $\mu\text{g}/\text{mL}$ overnight at 4C. Cells were stained counterstained with DAPI (blue). Cells were imaged using a 100X objective and digitally deconvolved.

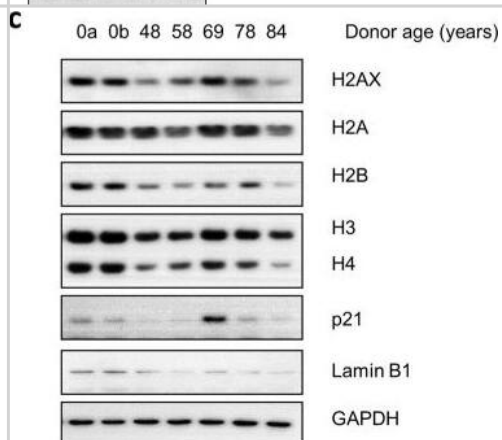


Western Blot: Histone H3 Antibody - BSA Free [NB500-171] - p53 but not ATM is required for chronic γ -radiation-induced histone reductions.

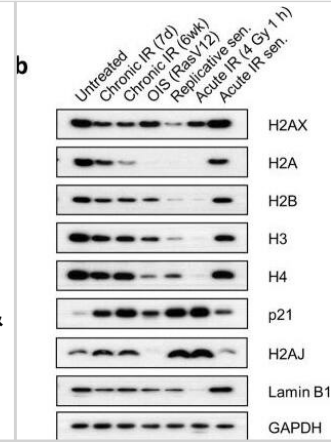
(a) Western blots of histone proteins in wild-type, TP53 $^{-/-}$ & ATM $^{-/-}$ RPE-1 cells following exposures to 20 mGy/h chronic radiation for 7 days. (b) ATM activation, as shown by auto-phosphorylation on S1981, in response to chronic radiation in primary fibroblasts. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32042076>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



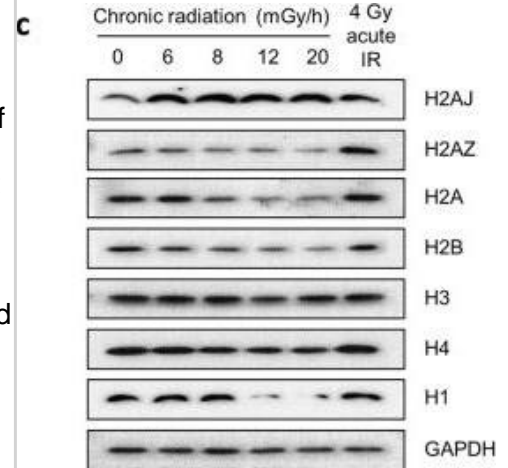
Western Blot: Histone H3 Antibody - BSA Free [NB500-171] - Reduced histone levels in senescent cells is induced in vitro by different means, & in vivo from aged donors. (a) Phase-contrast images of primary fibroblasts induced into senescence by chronic γ -radiation, oncogene over-expression or exhaustive replication (replicative senescence), & DNA damage from a single acute 4 Gy X-ray dose at an early time-point (1 hour) as a control for DNA damage without senescence. Scale bars 200 μm . (b) Western immunoblot analyses of histones in fibroblasts described in (a). (c) Histone levels in dermal fibroblasts isolated from human neonatal (age 0, donors a & b) & adult donors. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32042076>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Histone H3 Antibody - BSA Free [NB500-171] - Reduced histone levels in senescent cells is induced in vitro by different means, & in vivo from aged donors. (a) Phase-contrast images of primary fibroblasts induced into senescence by chronic γ -radiation, oncogene over-expression or exhaustive replication (replicative senescence), & DNA damage from a single acute 4 Gy X-ray dose at an early time-point (1 hour) as a control for DNA damage without senescence. Scale bars 200 μ m. (b) Western immunoblot analyses of histones in fibroblasts described in (a). (c) Histone levels in dermal fibroblasts isolated from human neonatal (age 0, donors a & b) & adult donors. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32042076>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Histone H3 Antibody - BSA Free [NB500-171] - Chronic γ -radiation reduces histone levels. (a) Western blot analyses of histone H2AX & γ H2AX in primary fibroblasts exposed to various dose-rates of chronic γ -radiation for 7 days. Cells irradiated with a single acute dose of 4 Gy X-ray were included as control. (b) Effect of chronic γ -irradiation on H2AX levels in three different isogenic primary cell types from a different donor to that used in (a) at the same dose rates as in (a). (c) Immunoblots of other histones in chronically irradiated primary fibroblasts. (d) All significant histone level changes detected by SILAC LC-MS/MS protein analyses of samples from primary fibroblasts exposed or mock-exposed to chronic γ -radiation for 7 days. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32042076>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Tosi GM, Regoli M, Altera A et al. Heat Shock Protein 90 Involvement in the Development of Idiopathic Epiretinal Membranes Investigative Ophthalmology & Visual Science 2020-07-28 [PMID: 32716502]

Soyal SM, Bonova P, Kwik M et al. The Expression of CNS-Specific PPARGC1A Transcripts Is Regulated by Hypoxia and a Variable GT Repeat Polymorphism Mol. Neurobiol. 2019-08-30 [PMID: 31471878]

Haiquan Lu, Yajing Lyu, Linh Tran, Jie Lan, Yangyiran Xie, Yongkang Yang, Naveena L Murugan, Yueyang J Wang, Gregg L Semenza HIF-1 recruits NANOG as a coactivator for TERT gene transcription in hypoxic breast cancer stem cells. Cell reports 2022-02-10 [PMID: 34592152]

0 Erratum for the Research Article: "Lactate promotes endothelial-to-mesenchymal transition via Snail1 lactylation after myocardial infarction". Science advances 2023-12-22 [PMID: 38134287]

Burke JM, Ripin N, Ferretti MB et al. RNase L activation in the cytoplasm induces aberrant processing of mRNAs in the nucleus PLoS pathogens 2022-11-01 [PMID: 36318584]

Details:

Dilution used in WB 1:1500

J. Blaze, A. Navickas, H. L. Phillips, S. Heissel, A. Plaza-Jennings, S. Miglani, H. Asgharian, M. Foo, C. D. Katanski, C. P. Watkins, Z. T. Pennington, B. Javidfar, S. Espeso-Gil, B. Rostandy, H. Alwaseem, C. G. Hahn, H. Molina, D. J. Cai, T. Pan, W. D. Yao, H. Goodarzi, F. Haghghi, S. Akbarian Neuronal Nsun2 deficiency produces tRNA epitranscriptomic alterations and proteomic shifts impacting synaptic signaling and behavior Nature Communications 2021-08-13 [PMID: 34389722]

Chellini L, Scarfò M, Bonvissuto D et al. The DNA/RNA helicase DHX9 orchestrates the KDM2B-mediated transcriptional regulation of YAP1 in Ewing sarcoma Oncogene 2023-11-28 [PMID: 38017132]

Verdile V, Palombo R, Ferrante G et al. Dysregulation of alternative splicing underlies synaptic defects in familial Amyotrophic Lateral Sclerosis Progress in neurobiology 2023-09-20 [PMID: 37739207] (WB, Human)

Chellini L, Pieraccioli M, Sette C, Paronetto MP. The DNA/RNA helicase DHX9 contributes to the transcriptional program of the androgen receptor in prostate cancer Journal of Experimental & Clinical Cancer Research 2022-12-01 [PMID: 35590370] (Western Blot)

Malnar ?rnigoj M, ?er?ek U, Yin X et al. Phenylalanine-tRNA aminoacylation is compromised by ALS/FTD-associated C9orf72 C4G2 repeat RNA Nat Commun 2023-09-16 [PMID: 37717009] (Immunocytochemistry/ Immunofluorescence)

Ait Saada A, Costa AB, Lobachev KS. Characterization of canavanine-resistance of cat1 and vhc1 deletions and a dominant any1 mutation in fission yeast PLOS ONE 2022-05-31 [PMID: 35639710] (Western Blot)

Kabacik S, Lowe D, Cohen H et al. Isolation of five different primary cell types from a single sample of human skin STAR Protocols 2022-06-01 [PMID: 35586317] (Western Blot)

More publications at <http://www.novusbio.com/NB500-171>

Procedures

Western Blot protocol for Histone H3 Antibody (NB500-171)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
 2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
 3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
 4. Rinse the blot.
 5. Block the membrane using standard blocking buffer for at least 1 hour.
 6. Wash the membrane in wash buffer three times for 10 minutes each.
 7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
 8. Wash the membrane in wash buffer three times for 10 minutes each.
 9. Apply the diluted 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 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.
- Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin protocol for Histone H3 Antibody (NB500-171)

Immunohistochemistry-Paraffin Embedded Sections Protocol

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.

Staining:

1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.



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 NB500-171

NB800-PC1	HeLa Whole Cell Lysate
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.

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