# **Product Datasheet**

# Histone Deacetylase 4/HDAC4 Antibody - BSA Free NBP2-22151-0.1ml

Unit Size: 0.1 ml

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

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# NBP2-22151-0.1ml

Histone Deacetylase 4/HDAC4 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	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	130 kDa
Product Description	
Host	Rabbit
Gene ID	9759
Gene Symbol	HDAC4
Species	Human, Mouse, Rat, Primate
Immunogen	A synthetic peptide made to a C-terminal portion of the human HDAC4 protein (between residues 1000-1084). [UniProt P56524]
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1.0 ug/mL, Simple Western 1:100, Immunohistochemistry 1:300, Immunocytochemistry/ Immunofluorescence 1:500 - 1:1000, Immunohistochemistry-Paraffin 1:300
Application Notes	<ul> <li>This HDAC4 antibody is useful for Western blot, Immunocytochemistry/Immunofluorescence, and Immunohistochemistry on paraffin embedded sections. In Western blot a band was observed approx. 130 kDa. In ICC/IF nuclear and cytoplasmic staining was observed in HeLa cells. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.</li> <li>In Simple Western only 10 - 15 uL of the recommended dilution is used per data point.</li> <li>See Simple Western Antibody Database for Simple Western validation: Tested in NIH-3T3 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:100.</li> <li>Separated by Size-Wes, Sally Sue/Peggy Sue.</li> <li>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.</li> </ul>



#### Images

Western Blot: Histone Deacetylase 4/HDAC4 Antibody [NBP2-22151] -Expression of HDACs in murine optic nerve. Western blot analysis of HDAC proteins in E16, P5 and P30 murine optic nerve was performed to check the specificity of the antibodies used for the study. Blots were normalized to beta-TUBULIN. Densitometric graphs for each HDAC panel show quantification of protein levels expressed as fold-change from E16. p < 0.05 was considered statistically significant and represented by a single \*, whereas p < 0.005 is represented by \*\*. Bars show mean +/- SEM of three samples per timepoint. Note that the apparent increase in band intensities for some P5 samples reflect increased protein loading in those lanes. Image collected and cropped by CiteAb from the following publication (https://bmcdevbiol.biomedcentral.com/articles/10.1186/1471-213X-14-

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Immunocytochemistry/Immunofluorescence: Histone Deacetylase 4/HDAC4 Antibody [NBP2-22151] - HDAC4 antibody was tested in HeLa cells at a 1:100 dilution against DyLight 488 (Green) using a 40X objective. Alpha tubulin and nuclei were counterstained against DyLight 568 (Red) and DAPI (Blue), respectively.



 $\frac{HDAC4}{C} = \frac{SOU2/HDAC4}{C} = \frac{SOU2/HDAC4}{C}$ 

Developmenta

Stage



Immunohistochemistry: Histone Deacetylase 4/HDAC4 Antibody [NBP2-22151] - HDAC4 localization pattern in developing murine optic nerve. Optic nerve sections at P30 were triple-labeled with HDAC4 (red). Double-label of SOX2 and HDAC4. Composite image in M show z stacks of optic nerve at high magnification; horizontal yellow lines correspond the x axis plane and vertical yellow lines corresponds to the y axis. The x, z axis is shown at the bottom of each panel, while the y, z axis is shown to the right of the panel. Abbreviations: R; retina, ON; optic nerve. Scale bars = 50 um. Image collected and cropped by CiteAb from the following publication

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Western Blot: Histone Deacetylase 4/HDAC4 Antibody [NBP2-22151] - Analysis of HDAC4 in NIH/3T3 lysate.



Immunohistochemistry: Histone Deacetylase 4/HDAC4 Antibody [NBP2-22151] - Analysis of HDAC4 in mouse pancreas.



Simple Western: Histone Deacetylase 4/HDAC4 Antibody [NBP2-22151] - Image shows a specific band for HDAC4 in 0.5 mg/mL of NIH-3T3 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

#### **Publications**

Zhao P, Xia W, Wei J et al. An Investigation of the Mechanisms of Radiation-Induced Muscle Injury in a Tree Shrew (Tupaia belangeri) Model Dose-Response 2022-01-01 [PMID: 35360454] (IF/IHC, Transgenic Mouse)

Wei D, Lu T, Ma D et al. Synergistic activity of imatinib and AR-42 against chronic myeloid leukemia cells mainly through HDAC1 inhibition. Life Sci. 2018-09-21 [PMID: 30248347] (WB, Human)

Pan P, Oshima K, Huang YW et al. Loss of FFAR2 promotes colon cancer by epigenetic dysregulation of inflammation suppressors. Int. J. Cancer. 2018-03-09 [PMID: 29524208] (Mouse)

Pan P, Skaer CW, Wang HT et al. Loss of Free Fatty Acid Receptor 2 enhances colonic adenoma development and reduces the chemopreventive effects of black raspberries in ApcMin/+ mice. Carcinogenesis. 2016-11-19 [PMID: 27866157]

Tiwari S, Dharmarajan S, Shivanna M et al. Histone deacetylase expression patterns in developing murine optic nerve. BMC Dev Biol 2014-07-09 [PMID: 25011550]



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#### **Procedures**

Western blot protocol for HDAC4 antibody (NBP2-22151) Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 25 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 anti-HDAC4 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 HDAC4 antibody (NBP2-22151) 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.

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.

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14. Dehydrate sections.

15. Mount coverslips.

Immunocytochemistry/Immunofluorescence protocol for HDAC4 antibody (NBP2-22151) Immunocytochemistry Protocol

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 NBP2-22151-0.1ml

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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