

Product Datasheet

HDAC6 Antibody - BSA Free

NB100-56343

Unit Size: 0.1 mg

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

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NB100-56343

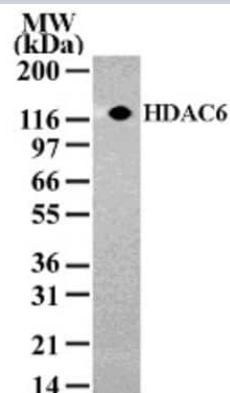
HDAC6 Antibody - BSA Free

Product Information	
Unit Size	0.1 mg
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
Product Description	
Host	Rabbit
Gene ID	10013
Gene Symbol	HDAC6
Species	Human, Mouse
Immunogen	Synthetic peptide made to an N-terminal portion of human HDAC6 (between amino acids 1-50) [UniProt Q9UBN7]
Product Application Details	
Applications	Western Blot, Simple Western, Chromatin Immunoprecipitation, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 2 - 5 ug/ml, Simple Western 1:20, Chromatin Immunoprecipitation 1:20-1:1000. Use reported in scientific literature (Imbriano (2005)), Immunocytochemistry/ Immunofluorescence 2 - 5 ug/ml, Immunoprecipitation assay dependent, Chromatin Immunoprecipitation (ChIP) 1:20-1:1000
Application Notes	In NIH 3T3, a 134 kDa band is observed. 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 NIH-3T3 lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:10, apparent MW was 131 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

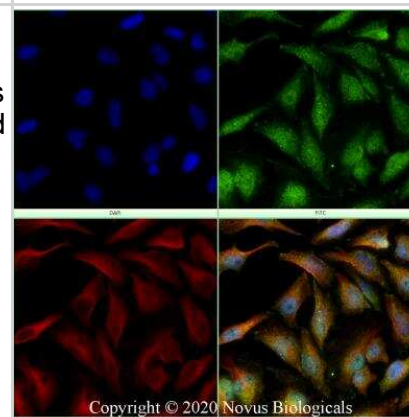


Images

Western Blot: HDAC6 Antibody [NB100-56343] - Analysis of HDAC-6 in NIH-3T3 cell lysate with this antibody.



Immunocytochemistry/Immunofluorescence: HDAC6 Antibody [NB100-56343] - HeLa cells were fixed for 10 minutes using 4% PFA and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-HDAC6 at 2 ug/ml overnight at 4C and detected with an anti-rabbit Dylight 488 (Green) at a 1:500 dilution. Alpha tubulin (DM1A) NB100-690 was used as a co-stain at a 1:1000 dilution and detected with an anti-mouse Dylight 550 (Red) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Simple Western: HDAC6 Antibody [NB100-56343] - Lane view shows a specific band for HDAC6 in 0.5 mg/ml of NIH-3T3 lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Publications

Bandolik JJ, Hamacher A, Schrenk C et al. Class I-Histone Deacetylase (HDAC) Inhibition is Superior to pan-HDAC Inhibition in Modulating Cisplatin Potency in High Grade Serous Ovarian Cancer Cell Lines International Journal of Molecular Sciences 2019-06-22 [PMID: 31234549] (WB, Human)

Imbriano C, Gurtner A, Cocchiarella F et al. Direct p53 transcriptional repression: in vivo analysis of CCAAT-containing G2/M promoters. Mol Cell Biol. 2005-05-01 [PMID: 15831478]

Procedures

Western Blot Protocol for HDAC6 Antibody (NB100-56343)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST 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 manufacturer's instructions.

Immunocytochemistry/ Immunofluorescence Protocol for HDAC6 Antibody (NB100-56343)

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeabilize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeabilization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPI if required.





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Products Related to NB100-56343

NB800-PC8	NIH 3T3 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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