Product Datasheet

KAT3B/p300 Antibody - BSA Free
NB500-161

Unit Size: 100 ul

Store at 4C. Do not freeze.

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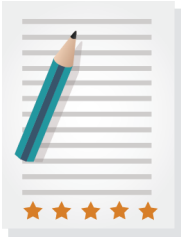
Updated 2/21/2025 v.20.1



NB500-161

KAT3B/p300 Antibody - BSA Free

Product Information

Unit Size	100 ul
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)

Product Description

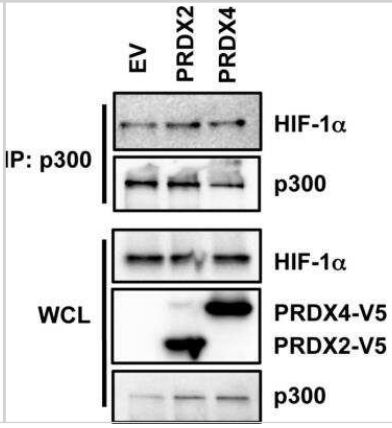
Host	Rabbit
Gene ID	2033
Gene Symbol	EP300
Species	Human, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID:33106900).
Immunogen	The immunogen this antibody was made to, maps to a region between residues 1000 and 1050 of human E1A-associated protein p300 using the numbering given in entry NP_001420.2 (GeneID 2033).

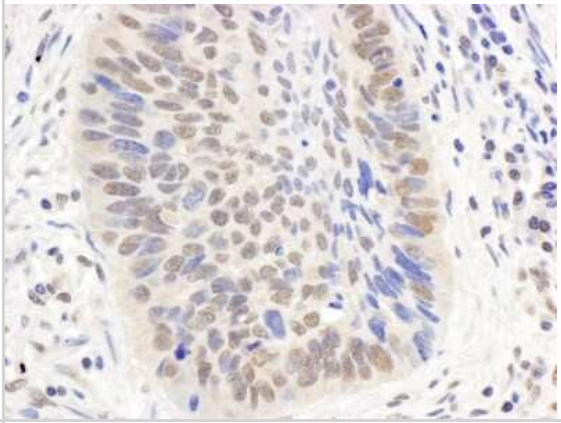
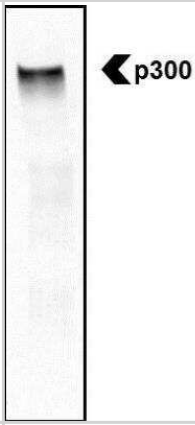
Product Application Details

Applications	Western Blot, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:1000-1:10000, Immunohistochemistry 1:500 to 1:2000, Immunoprecipitation 2-10 ug/mg of lysate, Immunohistochemistry-Paraffin 1:500 to 1:2000, Chromatin Immunoprecipitation (ChIP) 1:10-1:500
Application Notes	Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE tissue sections. KAT3B/p300 antibody validated for chip from a verified customer review.

Images

Western Blot: KAT3B/p300 Antibody [NB500-161] - Effect of PRDX2 and PRDX4 on HIF-1alpha-p300 interaction. HeLa cells were transfected with empty vector (EV) or vector encoding PRDX2-V5 or PRDX4-V5, and exposed to 1% O2 for 24 h. WCL was subject to IP with anti-p300 antibody, followed by immunoblot assays using antibodies against HIF-1alpha, V5, and p300. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/abstract/7142), licensed under a CC-BY license.



Immunohistochemistry-Paraffin: KAT3B/p300 Antibody [NB500-161] - Section of human lung carcinoma. Antibody: Affinity purified rabbit anti-p300 used at a dilution of 1:5,000 (0.2ug/ml). Detection: DAB	
Western Blot: KAT3B/p300 Antibody [NB500-161] - Analysis of HeLa lysates using NB500-161. Image courtesy of Gregg Semenza - publication number 21620138 (PMID).	
Western Blot: KAT3B/p300 Antibody [NB500-161] - Detection of Human p300 by Western Blot. Samples: Whole cell lysate (50 ug) from HeLa, 293T, and Jurkat cells. Antibodies: Affinity purified rabbit anti-p300 antibody NB500-161 used for WB at 0.1 ug/ml. Detection: Chemiluminescence with an exposure time of 10 seconds.	
KAT3B/p300 Antibody [NB500-161] - Detection of human p300 by western blot of immunoprecipitates. Samples: Whole cell lysate (1 mg for IP; 20% of IP loaded) from HeLa cells. Antibodies: Affinity purified rabbit anti-p300 antibody NB500-161 used for IP at 6 ug/mg lysate. p300 was also immunoprecipitated by a previous lot (lot NB500-161-1) of this antibody. For blotting immunoprecipitated p300, NB500-161 was used at 1 ug/ml. Detection: Chemiluminescence with an exposure time of 3 seconds.	

Publications

Salman S, Meyers DJ, Wicks EE Et al. HIF inhibitor 32-134D eradicates murine hepatocellular carcinoma in combination with anti-PD1 therapy J Clin Invest 2022-05-02 [PMID: 35499076] (Chemotaxis, Human)

Details:
Citation using the Alexa Fluor 488 version of this antibody.

Bao L, Chen Y, et al. Methylation of hypoxia-inducible factor (HIF)-1 alpha by G9a/GLP inhibits HIF-1 transcriptional activity and cell migration. Nucleic Acids Res 2018-07-27 [PMID: 29860315] (Chemotaxis, IP, Human)

Storchova R, Burdova K, Palek M et al. A novel assay for screening WIP1 phosphatase substrates in nuclear extracts The FEBS journal 2021-05-13 [PMID: 33982878]

Li B, Yu Y, Liu K et al. beta-Hydroxybutyrate inhibits histone deacetylase 3 to promote claudin-5 generation and attenuate cardiac microvascular hyperpermeability in diabetes Diabetologia 2020-10-27 [PMID: 33106900] (Rat)

Latorre-Muro P, Baeza J, Armstrong EA et al. Dynamic Acetylation of Phosphoenolpyruvate Carboxykinase Toggles Enzyme Activity between Gluconeogenic and Anaplerotic Reactions. Mol. Cell 2018-09-06 [PMID: 30193097] (WB, Human)

Lee MC, Huang HJ, Chang TH et al. Genome-wide analysis of HIF-2alpha chromatin binding sites under normoxia in human bronchial epithelial cells (BEAS-2B) suggests its diverse functions. Sci Rep 2016-07-04 [PMID: 27373565] (WB)

Luo W, Chen I, Chen Y et al. PRDX2 and PRDX4 are negative regulators of hypoxia-inducible factors under conditions of prolonged hypoxia Oncotarget. 2016-02-09 [PMID: 26837221] (WB)

Details:
The mechanism for feedback inhibition of hypoxia-inducible factors during prolonged hypoxia is clarified through studying the interaction of PRDX2 and PRDX4 with HIF-1 alpha and HIF-2 alpha.

Julien LA, Carriere A, Moreau J et al. mTORC1-activated S6K1 phosphorylates Rictor on threonine 1135 and regulates mTORC2 signaling. Mol Cell Biol 2010-02-01 [PMID: 19995915]

Luo W, Hu H, Chang R et al. Pyruvate Kinase M2 is a PHD3-Stimulated Coactivator for Hypoxia-Inducible Factor 1. Cell;145(5):732-44. 2011-05-27 [PMID: 21620138]

Procedures

Protocol specific for KAT3B / p300 Antibody (NB500-161)
Nuclear Extract and Cytoplasmic Fraction Preparation protocol for KAT3B / p300 Antibody (NB500-161):
Nuclear Extract and Cytoplasmic Fraction Preparation

1. Nuclear extracts (NE) and cytoplasmic fractions (S100) were prepared by Dignam's method (Dignam, Lebovitz, and Roeder, Nucleic Acids Res. 11: 1475-1489. 1983).
2. 100 liters of HeLa cell culture were harvested and washed 3 times with cold PBS.
3. The packed-cell volume (PCV) was measured, and the cell pellet was gently resuspended with 5 PCVs of hypotonic buffer (10 mM HEPES-KOH [pH 8], 10 mM KCl, 1.5 mM MgCl2, 1 mM DTT, 0.2 mM PMSF).
4. Cells were incubated on ice for 10 minutes and then pelleted by centrifugation at 1,800xg for 10 minutes.
5. Hypotonic buffer was added to 2 PCVs, and cells were resuspended and then homogenized with 15 strokes using a pestle B in a Dounce glass homogenizer until the cells were more than 90% lysed, as determined by a light microscope.
6. The lysate was centrifuged at 20,000xg for 30 minutes at 4 degrees Celcius.
7. The supernatant was saved for S100 fraction, and the pellet was saved to measure the packed nuclear volume (PNV).
8. 0.4 ml of extraction buffer (20 mM HEPES-KOH [pH 8], 0.6 M KCl, 1.5 mM MgCl2, 0.2 mM EDTA, 25% [vol/vol] glycerol, 1 mM DTT, 0.2 mM PMSF) per ml of PNV was added.
9. Cell nuclei were homogenized with 10 strokes of pestle A in the homogenizer.
10. Suspension was stirred at 4 degrees Celcius for 30 minutes and centrifuged for 30 minutes at 20,000xg.
11. The supernatant (nuclear extract) was aliquotted for use.
12. The S100 fraction (resulting supernatant) was mixed with 0.11 volume of high-salt buffer (20 mM HEPES-KOH [pH 8], 1.2 M KCl, 1.5 mM MgCl2, 0.2 mM EDTA, 20% [vol/vol] glycerol, 1 mM DTT, 0.2 mM PMSF) and centrifuged at 100,000xg for 60 minutes at 4 degrees Celcius.
13. This supernatant was dialyzed for 2 hours at 4 degrees Celcius.
14. The sample was centrifuged for 30 minutes at 20,000xg and the supernatant (S100) was aliquotted for use.

Immunoprecipitation

Antibody characterization:

1. HeLa NE and S100 were diluted with 1 volume of RIPA buffer [150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris [pH 8]).
2. Cleared by spinning at 100,000 g for 20 minutes at 4 degrees Celcius.
3. 1 ml of supernatant (~10 mg total protein) was mixed with 20 ug of primary antibody (NB 500-161) and rotated overnight at 4 degrees Celcius.
4. Supernatant was mixed with 0.05 ml of protein A-sepharose beads (50% slurry) and rotated for 2 hours at 4 degrees Celcius.
5. Immunoprecipitates were washed 3 times with the 10% RIPA in PBS.
6. The washed beads were boiled with 0.04 ml of Laemmli buffer and subjected to SDS-PAGE (4-20% Tris-glycine gel).

Complex purification:

1. NE and S100 were cleared by spinning at 20,000 g for 30 minutes at 4 degrees Celcius.
2. 1.5 ml of supernatant (~15 mg total protein) was mixed with 20 ug of primary antibody (NB 500-161) and rotated for 4 hours at 4 degrees Celcius.
3. Sample and antibody mixture were centrifuged at 15,000 g for 20 minutes at 4 degrees Celcius.
4. Supernatant was mixed with 0.05 ml of protein A-sepharose beads (50% slurry) and rotated for 1 hour at 4 degrees Celcius.
5. Immunoprecipitates were washed 3 times with the NETN buffer (20 mM Tris-HCl [pH 8], 100 mM NaCl, 1 mM EDTA, 0.5% NP-40).
6. The washed beads were boiled with 0.04 ml of Laemmli buffer and subjected to SDS-PAGE (4-20% Tris-glycine gel).

*If an insufficient amount of protein is purified for identification from 15 mg of extract, carry out the same procedure using 50-100 mg of extract to increase the amount of purified protein yield.

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Products Related to NB500-161

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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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