Product Datasheet

KAT3B/p300 Antibody (RW105) - BSA Free
NB100-616

Unit Size: 0.1 mg

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

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Publications: 13

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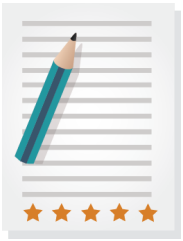
Updated 10/23/2024 v.20.1



NB100-616

KAT3B/p300 Antibody (RW105) - BSA Free

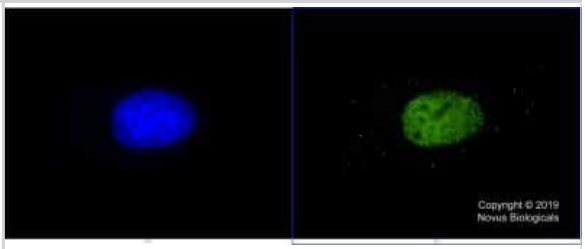
Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	RW105
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	2033
Gene Symbol	EP300
Species	Human, Mouse, Rat, Mustelid, Primate
Reactivity Notes	Mink.
Specificity/Sensitivity	This is specific for p300 protein. This recognizes residues 1921-2023.
Immunogen	Fusion protein containing residues 1572-2371 of human KAT3B/p300. [UniProt# Q0947]

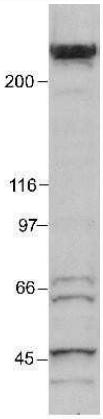
Product Application Details	
Applications	Western Blot, Electron Microscopy, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, In vitro assay, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:250-1:500, Flow Cytometry, Immunocytochemistry/ Immunofluorescence 1:50-1:200, Immunoprecipitation 1:10-1:500, In vitro assay reported in scientific literature (PMID 25728767), Electron Microscopy reported in scientific literature (PMID 25728767), Flow (Intracellular) 1ug/mL, Chromatin Immunoprecipitation (ChIP), Knockdown Validated Validated for Knockdown from a verified customer review.

Images

Immunocytochemistry/Immunofluorescence: KAT3B/p300 Antibody (RW105) [NB100-616] - HeLa cells were fixed in 4% paraformaldehyde for 10 min and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-KAT3B Antibody (RW105)) at 5ug/ml for 60 minutes at room temperature and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective.

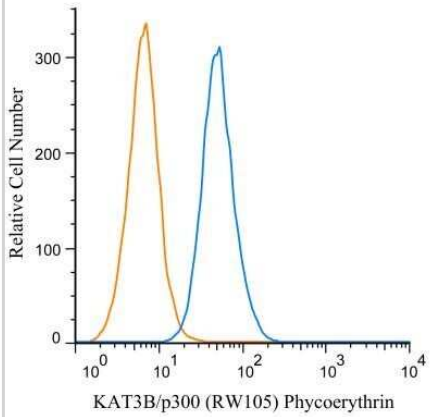


Western Blot: KAT3B/p300 Antibody (RW105) [NB100-616] - p300 detected in a HeLa nuclear extract using NB 100-616 (1:250). ECL: 20 minute exposure.

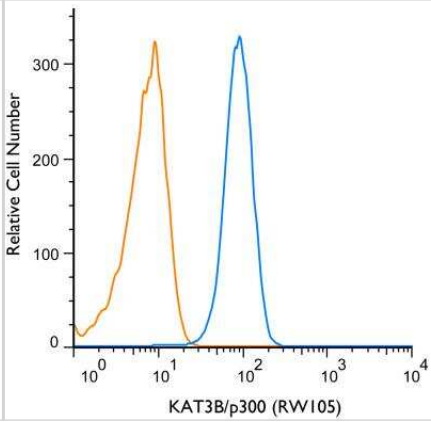


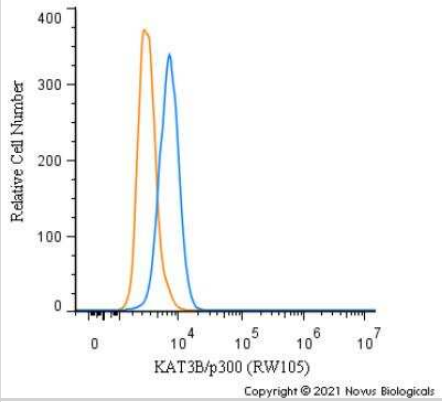
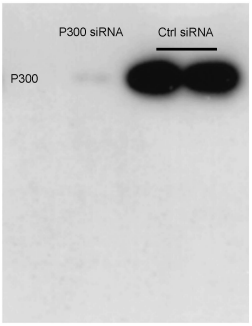
Immunocytochemistry/Immunofluorescence: KAT3B/p300 Antibody (RW105) [NB100-616] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.5% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-KAT3B/p300 Antibody (RW105) NB100-616 at 2 ug/ml overnight at 4C and detected with an anti-mouse 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.

Flow Cytometry: KAT3B/p300 Antibody (RW105) [NB100-616] - An intracellular stain was performed on RAW 246.7 cells with KAT3B/p300 antibody (RW105) NB100-616PE (blue) and a matched isotype control NBP2-27287PE (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 2 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin. Image using the PE format of this antibody.



Flow (Intracellular): KAT3B/p300 Antibody (RW105) [NB100-616] - An intracellular stain was performed on THP-1 cells with KAT3B/p300 (RW105) antibody NB100-616 (blue) and a matched isotype control NBP2-27287 (orange). Cells were fixed with 4% PFA and permeabilized with 0.1% Saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by mouse F(ab)2 IgG (H+L) APC-conjugated secondary antibody (F0101B, R&D Systems).



Flow Cytometry: KAT3B/p300 Antibody (RW105) [NB100-616] - An intracellular stain was performed on Raw264.7 cells with KAT3B/p300 Antibody (RW105) NB100-616 (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 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).	
Knockdown Validated: KAT3B/p300 Antibody (RW105) [NB100-616] - 293T cells were treated with different siRNAs against p300 alone for 2 days and core histones were prepared and subjected to WB analysis as indicated. The knockdown efficiency of p300 was confirmed by WB analysis of cell lysates. Western Blot protocol is 6% gel for running, 40V overnight transfer membrane. primary Ab 1:500, secondary Ab 1:5000. Image from verified customer review.	

Publications

Britta Kunkemoeller, Kuangyang Chen, Sam M Lockhart, Xuanchun Wang, Christian Rask-Madsen The transcriptional coregulator CITED2 suppresses expression of IRS-2 and impairs insulin signaling in endothelial cells. American journal of physiology. Endocrinology and metabolism 2021-09-20 [PMID: 34151583]

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]

Javadi S, Li Y, Shen J et al. Sustained correction of hippocampal neurogenic and cognitive deficits after a brief treatment by Nutlin-3 in a mouse model of Fragile X Syndrome BMC Med 2022-05-13 [PMID: 35549943]

Qin YP, Yu HB, Yuan SY et al. KAT2A Promotes Hepatitis B Virus Transcription and Replication Through Epigenetic Regulation of cccDNA Minichromosome Frontiers in Microbiology 2022-01-24 [PMID: 35140694]

Dhar SS, Zhao D, Lin T et al. MLL4 Is Required to Maintain Broad H3K4me3 Peaks and Super-Enhancers at Tumor Suppressor Genes Mol. Cell 2018-06-07 [PMID: 29861161] (Chemotaxis, Mouse)

He M, Zheng B, Zhang Y et al. KLF4 mediates the link between TGF-beta1-induced gene transcription and H3 acetylation in vascular smooth muscle cells. FASEB J 2015-09-01 [PMID: 26082460]

Yi P, Wang Z, Feng Q et al. Structure of a biologically active estrogen receptor-coactivator complex on DNA Mol. Cell 2015-03-19 [PMID: 25728767] (EM, In-vitro)

Byun SW, Chang YJ, Chung IS et al. Helicobacter pylori decreases p27 expression through the delta opioid receptor-mediated inhibition of histone acetylation within the p27 promoter Cancer Lett 2012-08-03 [PMID: 22867947] (Chemotaxis, Human)

Nemethova M, Wintersberger E. Polyomavirus large T antigen binds the transcriptional coactivator protein p300. J Virol;73(2):1734-9. 1999-02-01 [PMID: 9882390] (WB, IP, Mouse)

Eckner R et al. Association of p300 and CBP with simian virus 40 large T antigen. Mol Cell Biol;16(7):3454-64. 1996-07-01 [PMID: 8668161] (IP, Mouse)

Bhattacharya S, Michels CL, Leung MK, Arany ZP, Kung AL, Livingston DM. Functional role of p35srj, a novel p300/CBP binding protein, during transactivation by HIF-1. Genes Dev;13(1):64-75. 1999-01-01 [PMID: 9887100]

Eckner R et al. Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation. Genes Dev;1 (19):2478-90. 1996-10-01 [PMID: 8843199]

More publications at http://www.novusbio.com/NB100-616

Procedures

Flow (Intracellular) Protocol for KAT3B/p300 Antibody (NB100-616)
Protocol for Flow Cytometry Intracellular Staining
Sample Preparation.
1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.
a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.
4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 1 mL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeablization steps might reduce the availability of surface antigens.

Intracellular Staining.
Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.
Protocol for Cytoplasmic Targets:
Optional: Perform cell surface staining as described in the previous section.
1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.
a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.
b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).
3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 5 minutes at 400 RCF.
5. Discard supernatant and re-suspend in 1 mL of staining buffer + 0.1% permeabilizer.
6. Stain each sample at 1 uL/ 1 x 106 cells of primary antibody or 1-3 uL/ 1 x 106 cells for directly conjugated antibodies. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
7. Following the primary/conjugate incubation, add 2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 5 minutes at 400 RCF.
8. Remove supernatant and re-suspend each sample in 2 mL staining buffer + 0.1% permeabilizer, repeat wash for 5 minutes at 400 RCF.
9. If using a directly conjugated antibody, after the second wash, re-suspend cell pellet to a final volume of 500 uL per sample and proceed with flow analysis.

Western Blot Protocol for KAT3B/p300 Antibody (NB100-616)
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.

Immunodiffusion Protocol for KAT3B/p300 Antibody (NB100-616)
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. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization 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 NB100-616

NB800-PC9	HeLa Nuclear Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)

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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