Product Datasheet

KAT3B/p300 Antibody (RW128) - BSA Free
NB100-507

Unit Size: 0.1 mg

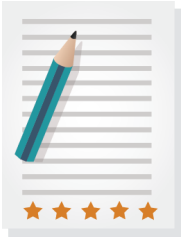
Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

www.novusbio.com technical@novusbio.com

Publications: 7

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-507

Updated 9/9/2025 v.20.1



NB100-507

KAT3B/p300 Antibody (RW128) - 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	RW128
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein A purified
Buffer	PBS
Target Molecular Weight	300 kDa

Product Description

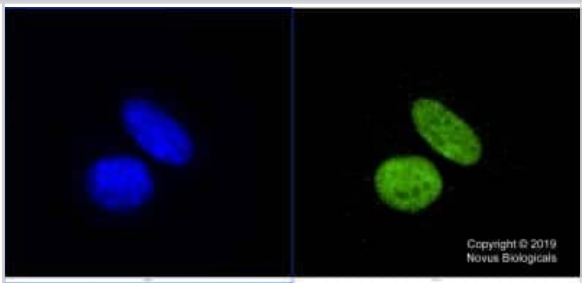
Description	Novus Biologicals Mouse KAT3B/p300 Antibody (RW128) - BSA Free (NB100-507) is a monoclonal antibody validated for use in WB, Flow, ICC/IF and IP. Anti-KAT3B/p300 Antibody: Cited in 7 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Mouse
Gene ID	2033
Gene Symbol	EP300
Species	Human, Mouse, Rat, Mustelid, Primate
Immunogen	Fusion protein containing residues 1572-2371 of human KAT3B/p300. [UniProt# Q0947]

Product Application Details

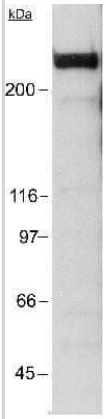
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunoprecipitation
Recommended Dilutions	Western Blot 1:250-1:500, Flow Cytometry, Immunocytochemistry/ Immunofluorescence 2-5ug/mL, Immunoprecipitation 1:10-1:500, Flow (Intracellular) 1ug/mL

Images

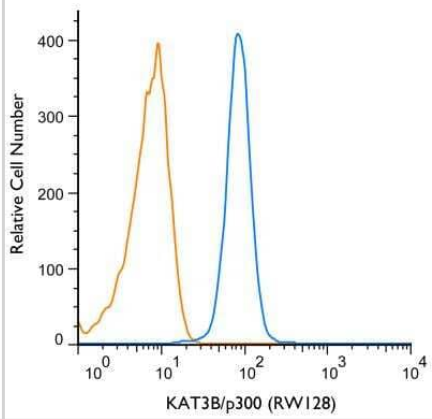
Immunocytochemistry/Immunofluorescence: KAT3B/p300 Antibody (RW128) [NB100-507] - HeLa cells were fixed in 4% paraformaldehyde for 10 min and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-KAT3B Antibody (RW128) at 5 ug/ml for 60 minutes at room temperature 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.



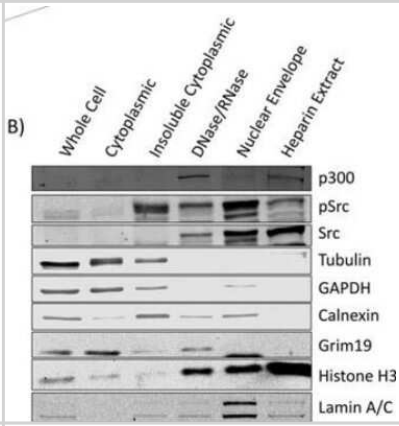
Western Blot: KAT3B/p300 Antibody (RW128) [NB100-507] - Detection of p300 in a HeLa nuclear extract using NB100-507 (1:250). ECL: 15 sec exposure.



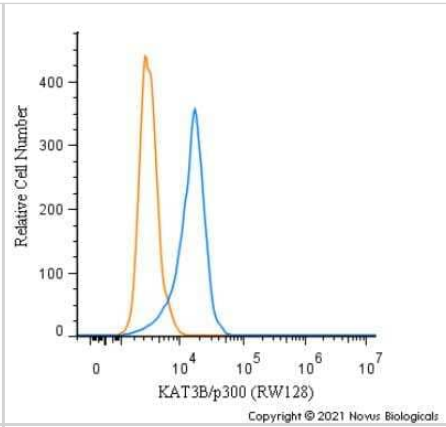
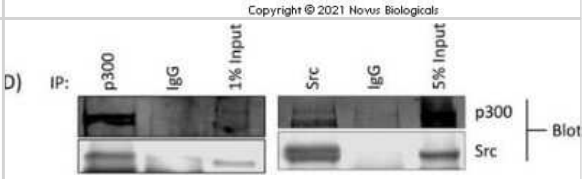
Flow (Intracellular): KAT3B/p300 Antibody (RW128) [NB100-507] - An intracellular stain was performed on THP-1 cells with KAT3B/p300 (RW128) antibody NB100-507 (blue) and a matched isotype control NBP2-27287 (orange). Cells were fixed with 4% PFA and permeabilized with 0.1% Saponin. Cells were incubated 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).



Western Blot: KAT3B/p300 Antibody (RW128) [NB100-507] - Western Blot: KAT3B/p300 Antibody (RW128) [NB100-507] - Kinase active Src is present in the nuclei of Panc-1 cells in association with p300. Subcellular fractionation and localization of Src and p300 in Panc-1 cells. Cell lysates of equal total protein or Panc-1 subcellular fractions were subjected to immunoblotting analysis for Src, pSrc, p300, tubulin (cytoskeletal proteins), GAPDH (soluble cytoplasmic proteins), calnexin (endoplasmic reticulum), grim19 (mitochondria), histone H3 (chromatin), and lamin A/C (nuclear lamina). Image collected and cropped by Citeab from the following publication (A novel nuclear Src and p300 signaling axis controls migratory and invasive behavior in pancreatic cancer. *Oncotarget* (2016) licensed under a CC-BY license.



Immunocytochemistry/Immunofluorescence: KAT3B/p300 Antibody (RW128) [NB100-507] - 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 (RW128) NB100-507 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 (RW128) [NB100-507] - An intracellular stain was performed on Raw264.7 cells with KAT3B/p300 Antibody (RW128) NB100-507 (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).	
Western Blot: KAT3B/p300 Antibody (RW128) [NB100-507] - Kinase active Src is present in the nuclei of Panc-1 cells in association with p300. Src and p300 co-association in Panc-1 cells. Immunoblotting analysis of Src or p300 immunecomplexes from Panc-1 nuclear isolates probing for Src or p300, or IgG control. Image collected and cropped by Citeab from the following publication (A novel nuclear Src and p300 signaling axis controls migratory and invasive behavior in pancreatic cancer. Oncotarget (2016) licensed under a CC-BY license.	

Publications

Kung A L, Zabludoff S D et al. Small molecule blockade of transcriptional coactivation of the hypoxia-inducible factor pathway. Cancer Cell 2004-01-07 [PMID: 15261140] (WB, Human)

Paladino David, Yue Peibin, Furuya Hideki et al. A novel nuclear Src and p300 signaling axis controls migratory and invasive behavior in pancreatic cancer. Oncotarget 2016-01-01 [PMID: 26695438] (WB, 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]

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]

Eckner R et al. Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. Genes Dev;8(8):869-84. 1994-04-15 [PMID: 7523245]

Procedures

Western Blot protocol for KAT3B/p300 Antibody (NB100-507)
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.

Flow (Intracellular) protocol for KAT3B/p300 Antibody (NB100-507)

KAT3B/p300 Antibody (RW128):

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 100 uL 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:

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 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Immunocytochemistry/Immunofluorescence protocol for KAT3B/p300 Antibody (NB100-507)
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.

Flow Cytometry Protocol for KAT3B/p300 Antibody (NB100-507)

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.

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 NB100-507

NB800-PC9	HeLa Nuclear Cell Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB100-507

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications