

Product Datasheet

NCOA3/AIB1 Antibody - BSA Free NB100-314

Unit Size: 100 ul

Store at 4C. Do not freeze.

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NB100-314

NCOA3/AIB1 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)

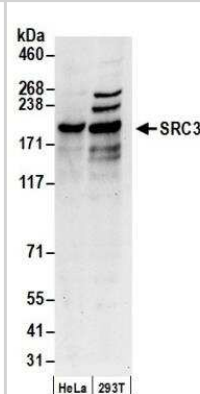
Product Description	
Description	This antibody can be used as the primary antibody in a PLA assay with the following as complementing antibodies:NB100-224, NB500-160, NB200-347, NBP1-06527, NBP1-19102
Host	Rabbit
Gene ID	8202
Gene Symbol	NCOA3
Species	Human
Immunogen	A synthetic peptide, which represented a portion of human nuclear receptor coactivator 3 encoded within exon 13 (LocusLink ID 8202).
Notes	This antibody can be used as the primary antibody in a PLA assay with the following as complementing antibodies:NB100-224, NB500-160, NB200-347, NBP1-06527, NBP1-19102

Product Application Details	
Applications	Western Blot, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000-1:10000, Immunoprecipitation 1-4 ug/mg lysate
Application Notes	NB 100-314 may be used for Western blot and immunoprecipitation. This protein is a cytoplasmic coactivator that shifts to the nucleus upon activation. It can be immunoprecipitated and analyzed on Western blot using nuclear or cytosolic extracts. Suggested working dilutions: * Western Blot - 1:1,000 ~ 1:10,000 (5 ug nuclear extract) Immunocytochemistry - Not Done Immunoprecipitation - 1-4 ug/mg lysate *The investigator should determine the optimal working dilution for a specific application.

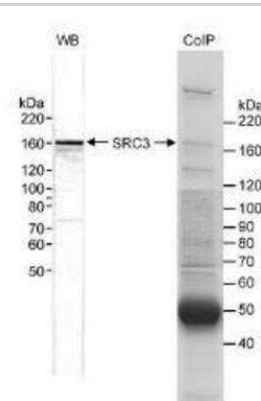


Images

Western Blot: NCOA3/AIB1 Antibody [NB100-314] - Detection of Human SRC3 by Western Blot. Samples: Whole cell lysate (50 ug) from HeLa and 293T cells prepared using NETN lysis buffer. Antibody: Affinity purified rabbit anti-SRC3 antibody NB100-314 used for WB at 0.1 ug/ml. Detection: Chemiluminescence with an exposure time of 30 seconds.



Western Blot: NCOA3/AIB1 Antibody [NB100-314] - Nuclear extract (5 mcg for WB, 10 mg for CoIP) from HeLa cells. Affinity purified rabbit anti-SRC3 used at 0.33 mcg/ml for WB and 20 mcg/10 mg extract for CoIP.



Publications

Lanz RB, Bulynko Y, Malovannaya A et al. Global characterization of transcriptional impact of the SRC-3 coregulator. *Mol Endocrinol* 2010-04-01 [PMID: 20181721]

Chen R, Liu M, Li H et al. PP2B and PP1alpha cooperatively disrupt 7SK snRNP to release P-TEFb for transcription in response to Ca²⁺ signaling. *Genes Dev* 2008-05-01 [PMID: 18483222]

Naeem H, Cheng D, Zhao Q et al. The activity and stability of the transcriptional coactivator p/CIP/SRC-3 are regulated by CARM1-dependent methylation. *Mol Cell Biol* 2007-01-01 [PMID: 17043108]

Li X, Lonard DM, Jung SY et al. The SRC-3/AIB1 coactivator is degraded in a ubiquitin- and ATP-independent manner by the REGgamma proteasome. *Cell* 2006-01-01 [PMID: 16439211]

Procedures

Protocol specific for AIB1 Antibody (NB100-314)

Nuclear Extract and Cytoplasmic Fraction Preparation protocol for AIB1 Antibody (NB100-314):

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 100-314) 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 100-314) 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.



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 NB100-314

NB800-PC9	HeLa Nuclear 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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