Product Datasheet

NCOA2 Antibody - BSA Free

NB100-388

Unit Size: 100 ul

Store at 4C. Do not freeze.

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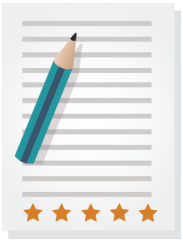
Updated 9/9/2025 v.20.1



NB100-388

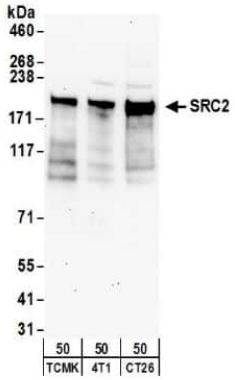
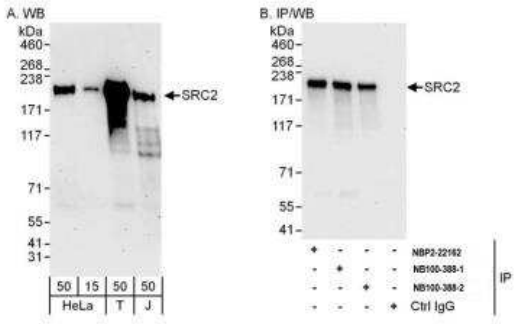
NCOA2 Antibody - BSA Free

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	Novus Biologicals Rabbit NCOA2 Antibody - BSA Free (NB100-388) is a polyclonal antibody validated for use in WB, ICC/IF and IP. Anti-NCOA2 Antibody: Cited in 1 publication. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	10499
Gene Symbol	NCOA2
Species	Human, Mouse, Rat
Reactivity Notes	Rat reactivity reported in scientific literature (PMID: 28552353).
Specificity/Sensitivity	Antibody was affinity purified using an epitope specific to SRC2 immobilized on solid support.
Immunogen	The immunogen recognized by this antibody maps to a region between residues 700 and 750 of human Nuclear Receptor Coactivator 2 using the numbering given in entry NP_006531.1 (GeneID 10499).

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000-1:10000, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation 1-4 ug/mg lysate
Application Notes	NB 100-388 may be used for Western Blot, where a band at ~160 kDa is seen, representing SRC2 protein. Though this antibody will recognize cytosolic protein in Western blot, it does much better with nuclear extracts. It may also be used for immunoprecipitation on HeLa nuclear extracts. Suggested working dilutions: * Western Blot - 1:1,000-1:10,000 Immunocytochemistry - Not Determined Immunoprecipitation - 1-4 ug/mg *The investigator should determine the optimal working dilution for a specific application. Use in Immunocytochemistry/immunofluorescence reported in scientific literature (PMID: 28552353).

Images

Western Blot: NCOA2 Antibody [NB100-388] - Detection of Mouse NCOA2/ SRC2 by Western Blot. Samples: Whole cell lysate (50 ug) from TCMK-1, 4T1, and CT26.WT cells. Antibodies: Affinity purified rabbit anti-NCOA2/SRC2 antibody NB100-388 used for WB at 1 ug/ml. Detection: Chemiluminescence with an exposure time of 3 minutes.	
Immunoprecipitation: NCOA2 Antibody [NB100-388] - Detection of human NCOA2/ SRC2 by western blot and immunoprecipitation. Samples: Whole cell lysate from HeLa(15 and 50 ug for WB; 1 mg for IP, 20% of IP loaded), HEK293T (T; 50 ug) and Jurkat (J; 50 ug) cells. Antibodies: Affinity purified rabbit anti-NCOA2/SRC2 antibody NB100-388 (lot 2) used for WB at 0.1 ug/ml (A) and 1 ug/ml (B) and used for IP at 6 ug/mg lysate. SRC2 was also immunoprecipitated by a previous lot (lot 1) and by rabbit anti-NCOA1/SRC2 antibody NBP2-22162, which recognizes a downstream epitope. Detection: Chemiluminescence with exposure times of 3 minutes (A) and 10 seconds (B).	

Publications

Long AF, Udy DB, Dumont S. Hec1 Tail Phosphorylation Differentially Regulates Mammalian Kinetochore Coupling to Polymerizing and Depolymerizing Microtubules Curr. Biol. 2017-06-05 [PMID: 28552353] (ICC/IF, Rat)

Procedures

Serum protocol for NCOA2 Antibody (NB100-388)
Nuclear Extract and Cytoplasmic Fraction Preparation protocol for NCOA2 Antibody (NB100-388):
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 100-388) 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 100-388) 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 NB100-388

NB800-PC9	HeLa Nuclear Cell Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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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