

Affinity-purified Goat Anti-human/mouse NCOA3 Antibody

ORDERING INFORMATION

Catalog Number: AF5076

Lot Number: CADH01

Size: 100 µg

Storage: -20° C

Specificity: human/mouse NCOA3

Immunogen: *E. coli*-derived recombinant human NCOA3 (rhNCOA3; aa 673 - 856)

Ig Type: affinity-purified goat IgG

Application: Western blot

Background

Nuclear Receptor Coactivator 3 (NCOA3) functions in the multisubunit coactivator complex. Multiple nuclear hormone receptors are able to bind to NCOA3 in a hormone dependent-manner. NCOA3 contains intrinsic histone acetyltransferase activity, but is also able to recruit other histone acetyltransferases (CBP, PCAF) and methyltransferases (PRMT1, CARM1) to active promoters. NCOA3 null mice exhibit hematological, adipogenesis, and endotoxic impairments. mRNA of NCOA3 is often amplified in several cancers including breast and ovarian.

Preparation

Goat antibodies were raised against purified, *E. coli*-derived, recombinant human NCOA3 (rhNCOA3; aa 673 - 856; Accession # NP_006525). Polyclonal antibody was affinity-purified on a column derivatized with rhNCOA3 and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) containing 0.02% trehalose.

Reconstitution

Reconstitute the antibody with 100 µL of sterile PBS containing 0.02% Na₃N.

Storage

The reconstituted antibody should be aliquoted and stored at -20° C in a manual defrost freezer for 12 months without detectable loss of activity. **Avoid repeated freeze/thaw cycles.**

Specificity

The antibody detects endogenous human and mouse NCOA3 in Western blots.

Application

Western blot - An antibody concentration of 0.1 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer

25 mM Tris, pH 7.5
0.15 M NaCl
0.05% Tween® 20

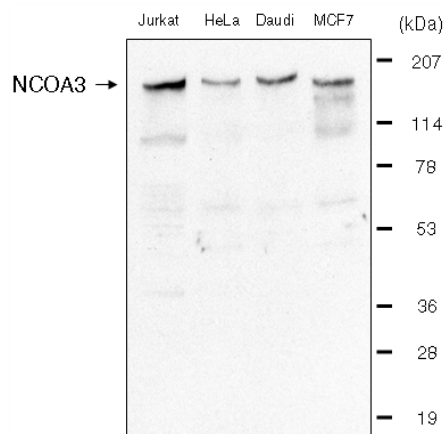
Blocking Solution

5% nonfat dry milk
in Blotting Buffer
Adjust pH to 7.5

1. Transfer the electrophoresed proteins onto a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane overnight at 2° - 8° C in Blocking Solution containing 0.1 µg/mL goat anti-NCOA3 antibody.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Blocking Solution containing a 1:2000 dilution of HRP-conjugated donkey anti-goat Ig (R&D Systems, Catalog # HAF109).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - A single plate (150 mm) of exponentially growing cells is washed twice in cold PBS. Cells are then removed from plates by scraping and pelleted by centrifugation. 300 - 500 µL of RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% Deoxycholate, 0.1% SDS, 50 mM Tris, pH 7.5) is added to cellular pellet (dependent upon cell type and volume). Cells are resuspended and incubated on ice for 5 minutes. The cells are then centrifuged at 15,000 rpm for 10 minutes. Supernatant is transferred to a new tube and quantified by Bradford analysis. 30 µg of cellular protein is added to an equal amount of 2x SDS loading buffer. Samples are then boiled for 5 minutes and run on a SDS page gel. Expected yield of protein 300 - 700 µg/150 mm plate of cells.

Optimal dilutions should be determined by each laboratory for each application.



10 µg of nuclear cell extracts from exponentially growing Jurkat, HeLa, Daudi, and MCF7 cells were prepared, resolved by SDS-PAGE, and transferred to a PVDF membrane. The membrane was immunoblotted with 0.1 µg/mL goat anti-NCOA3 antibody.