Affinity-purified Sheep Anti-human/mouse/rat NCOR1 Antibody

ORDERING INFORMATION

Catalog Number: AF6167

Lot Number: ZZF01

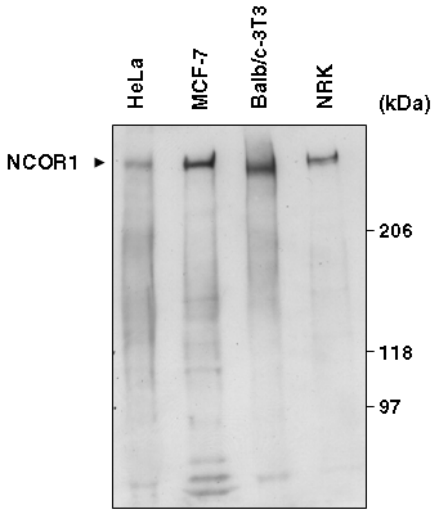
Size: 100 µg

Specificity: human, mouse and rat NCOR1

Immunogen: *E. coli*-derived rhNCOR1 (aa 1770 - 1947)

Ig Type: sheep IgG

Application: Western blot



Detection of NCOR1 with AF6167.
Lysates from human HeLa, MCF-7, mouse Balb/c-3T3 and rat NRK cells were resolved by SDS-PAGE. Following electrophoresis, lysates were transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL anti-NCOR1 as described in *Protocols for Immunoblotting*.

Background

NCOR1 (Nuclear receptor Co-Repressor 1) is a 270 kDa member of the NCoR family of molecules. It is widely expressed, being found in hepatocytes, intestinal crypt cells, neural stem cells, plus immature thymocytes and erythrocytes. NCOR1 is a transcriptional repressor. It forms a complex with HDAC3, TAB2 and ZBTB33, and interacts with a ligand-independent THR:RXR heterodimer bound to select gene promoters. Human NCOR1 is 2440 amino acids (aa) in length. It possesses one N-terminal repression domain (aa 1 - 312), two DNA-binding SANT domains (aa 437 - 674) and a second repression domain (aa 737 - 1004). Multiple Ser, Thr and Tyr phosphorylation sites exist that regulate complex dissociation. There are multiple potential splice variants. Short poly Lys motifs serve as substitutions for the C-terminal 1900 - 1910 amino acids. There is also a 16 aa insertion after Glu727, coupled to either a Ile substitution for aa 1842 - 1961, or a six aa substitution for aa 31 - 145. Over aa 1770 - 1947, human NCOR1 shares 96% aa identity with mouse NCOR1.

Preparation

Sheep antibodies were raised against purified, *E. coli*-derived recombinant human NCOR1 (rhNCOR1; aa 1770 - 1947; Accession # O75376). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein and further purified by isolating the IgG fraction.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute in PBS containing 0.02% NaN_3.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C **in a manual defrost freezer** for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects endogenous human, mouse and rat NCOR1 at 270 kDa using Western blot.

Application

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Protocols for Immunoblotting

Blotting Buffer	Blocking Solution	Antibody Solution
25 mM Tris, pH 7.4	5% nonfat dry milk	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer	in Blotting Buffer
0.1% Tween® 20	Adjust pH to 7.4	Adjust pH to 7.4

1. Transfer the electrophoresed proteins to an Immobilon-P membrane (Millipore) and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing 1.0 µg/mL sheep anti-NCOR1.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Antibody Solution containing a 1:5,000 dilution of HRP-conjugated donkey anti-sheep Ig (R&D Systems, Catalog # HAF016).
5. Wash the membrane for 1 hour with 5 or more changes of Blotting Buffer.
6. Detect with chemiluminescent detection reagents.

Cell lysates for Western blottings - To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF, and bromophenyl blue) at 2 x 10^6 - 1 x 10^7 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each.

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

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