

NEDD8 (E-16): sc-5479

BACKGROUND

NEDD8 is a ubiquitin (Ub)-like molecule that shares 80% homology with ubiquitin, a protein known to modify and target various proteins for proteolytic degradation. NEDD8 and the corresponding yeast homolog RUB1 are activated by the E1 ubiquitin activating enzyme UBA2, which forms isopeptide linkages between thio esters. Similar to the Ub-mediated proteolytic pathway, NEDD8 is covalently coupled to an E3 Ub ligase by the E2 conjugating enzyme, UBC12. NEDD8 preferentially associates with the CUL-2 protein in the E3 ligase complex CBCVHL, which consists of cullin proteins associating with elongin B/C and the tumor suppressor F-box protein von Hippel Lindau (VHL). NEDD8 is predominantly localized to the nucleus and is highly expressed in adult heart and skeletal muscle. *In vitro* studies indicate that NEDD8 tetramers are also able to bind to the 26S proteasome, and that they assemble into complexes with conserved Ub-like moieties, suggesting that NEDD8 may regulate proteolysis of intracellular proteins similar to other Ub-mediated pathways.

REFERENCES

1. Kamitani, T., et al. 1997. Characterization of NEDD8, a developmentally down-regulated ubiquitin-like protein. *J. Biol. Chem.* 272: 28557-28562.
2. Tanaka, K., et al. 1998. The ligation systems for ubiquitin and ubiquitin-like proteins. *Mol. Cells* 8: 503-512.
3. Whitby, F.G., et al. 1998. Crystal structure of the human ubiquitin-like protein NEDD8 and interactions with ubiquitin pathway enzymes. *J. Biol. Chem.* 273: 34983-34991.
4. Osaka, F., et al. 1998. A new NEDD8-ligating system for cullin-4A. *Genes Dev.* 12: 2263-2268.
5. Gong, L., et al. 1999. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. *J. Biol. Chem.* 274: 12036-12042.
6. Wada, H., et al. 1999. Identification of NEDD8-conjugation site in human cullin-2. *Biochem. Biophys. Res. Commun.* 257: 100-105.

CHROMOSOMAL LOCATION

Genetic locus: NEDD8 (human) mapping to 14q12.

SOURCE

NEDD8 (E-16) is an affinity purified goat polyclonal antibody raised against a peptide mapping at the N-terminus of NEDD8 of human origin.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-5479 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

NEDD8 (E-16) is recommended for detection of NEDD8 of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

NEDD8 (E-16) is also recommended for detection of NEDD8 in additional species, including equine, canine, bovine and porcine.

Suitable for use as control antibody for NEDD8 siRNA (h): sc-36026, NEDD8 shRNA Plasmid (h): sc-36026-SH and NEDD8 shRNA (h) Lentiviral Particles: sc-36026-V.

Molecular Weight of NEDD8 monomer: 6 kDa.

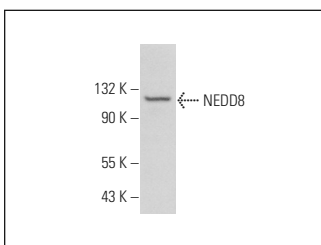
Molecular Weight of NEDD8-protein conjugate: 90 kDa.

Positive Controls: Raji whole cell lysate: sc-364236, HL-60 whole cell lysate: sc-2209 or K-562 whole cell lysate: sc-2203.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

DATA



NEDD8 (E-16): sc-5479. Western blot analysis of NEDD8 expression in Raji whole cell lysate.

RESEARCH USE

For research use only, not for use in diagnostic procedures.



Try **NEDD8 (H-2): sc-373741**, our highly recommended monoclonal alternative to NEDD8 (E-16).