

NEDD8 (H-2): sc-373741

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.

CHROMOSOMAL LOCATION

Genetic locus: NEDD8 (human) mapping to 14q12; Nedd8 (mouse) mapping to 14 C3.

SOURCE

NEDD8 (H-2) is a mouse monoclonal antibody raised against amino acids 1-81 representing full length NEDD8 of human origin.

PRODUCT

Each vial contains 200 µg IgG₁ kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

NEDD8 (H-2) is available conjugated to agarose (sc-373741 AC), 500 µg/0.25 ml agarose in 1 ml, for IP; to HRP (sc-373741 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-373741 PE), fluorescein (sc-373741 FITC), Alexa Fluor® 488 (sc-373741 AF488), Alexa Fluor® 546 (sc-373741 AF546), Alexa Fluor® 594 (sc-373741 AF594) or Alexa Fluor® 647 (sc-373741 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-373741 AF680) or Alexa Fluor® 790 (sc-373741 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

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STORAGE

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

APPLICATIONS

NEDD8 (H-2) is recommended for detection of NEDD8 of mouse, rat and human origin by Western Blotting (starting dilution 1:100, 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); partially cross reactive with Ubiquitin and other Ubiquitin-like proteins.

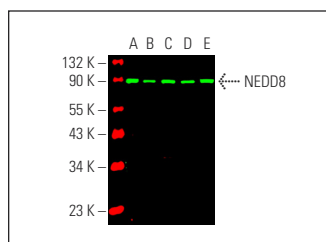
Suitable for use as control antibody for NEDD8 siRNA (h): sc-36026, NEDD8 siRNA (m): sc-36027, NEDD8 shRNA Plasmid (h): sc-36026-SH, NEDD8 shRNA Plasmid (m): sc-36027-SH, NEDD8 shRNA (h) Lentiviral Particles: sc-36026-V and NEDD8 shRNA (m) Lentiviral Particles: sc-36027-V.

Molecular Weight of NEDD8 monomer: 6 kDa.

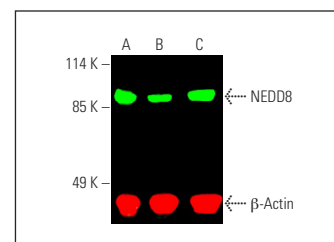
Molecular Weight of NEDD8-protein conjugate: 90 kDa.

Positive Controls: C6 whole cell lysate: sc-364373, Ramos cell lysate: sc-2216 or HL-60 whole cell lysate: sc-2209.

DATA



NEDD8 (H-2) Alexa Fluor® 680: sc-373741 AF680. Direct near-infrared western blot analysis of NEDD8 expression in HL-60 (A), BJAB (B), Ramos (C), THP-1 (D) and GA-10 (E) whole cell lysates. Blocked with UltraCruz® Blocking Reagent: sc-516214. Cruz Marker™ Molecular Weight Standards detected with Cruz Marker MW Tag-Alexa Fluor® 790: sc-516731.



Simultaneous direct near-infrared western blot analysis of NEDD8 expression, detected with NEDD8 (H-2) Alexa Fluor® 680: sc-373741 AF680 and beta-Actin expression, detected with beta-Actin (C4) Alexa Fluor® 790: sc-47778 AF790 in GA-10 (A), THP-1 (B) and C6 (C) whole cell lysates. Blocked with UltraCruz® Blocking Reagent: sc-516214.

SELECT PRODUCT CITATIONS

1. Balasubramaniam, B., et al. 2022. p38-MAPK recruits the proteolysis pathways in *Caenorhabditis elegans* during bacterial infection. *Int. J. Biol. Macromol.* 204: 116-135.
2. Borgo, C., et al. 2022. Targeting the E1 ubiquitin-activating enzyme (UBA1) improves elxacaftor/tezacaftor/ivacaftor efficacy towards F508del and rare misfolded CFTR mutants. *Cell. Mol. Life Sci.* 79: 192.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.