SANTA CRUZ BIOTECHNOLOGY, INC.

NEDD8 siRNA (m): sc-36027

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 downregulated 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. Cell 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.
7. Liakopoulos, D., et al. 1999. Conjugation of the ubiquitin-like protein NEDD8 to cullin-2 is linked to von Hippel-Lindau tumor suppressor function. Proc. Natl. Acad. Sci. USA 96: 5510-5515.

CHROMOSOMAL LOCATION

Genetic locus: Nedd8 (mouse) mapping to 14 C3.

PRODUCT

NEDD8 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NEDD8 shRNA Plasmid (m): sc-36027-SH and NEDD8 shRNA (m) Lentiviral Particles: sc-36027-V as alternate gene silencing products.

For independent verification of NEDD8 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36027A, sc-36027B and sc-36027C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NEDD8 siRNA (m) is recommended for the inhibition of NEDD8 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

NEDD8 (H-2): sc-373741 is recommended as a control antibody for monitoring of NEDD8 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NEDD8 gene expression knockdown using RT-PCR Primer: NEDD8 (m)-PR: sc-36027-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chadha, A., et al. 2015. Suppressive role of neddylation in dendritic cells during *Mycobacterium tuberculosis* infection. Tuberculosis 95: 599-607.

RESEARCH USE

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