

NEDD4 siRNA (r): sc-270215

BACKGROUND

NEDD4 family interacting protein-1 (NDFIP1), also known as N4WBP5, is a member of a family of highly conserved proteins. NEDD4 is a 221 amino acid, Golgi-associated protein which contains three transmembrane domains in its carboxy-terminus and two PY motifs in its amino-terminus. NEDD4 may play a role in Golgi structure and function. It binds the WW domains of a number of NEDD4 family members called HECT-type E3 ubiquitin ligases. NEDD4 is strongly expressed in surviving neurons around an injury site, which suggests that ubiquitination may be a possible survival strategy and that NEDD4 may act as a neuroprotective protein. It may also have an effect on the function of Itch, another E3 ubiquitin ligase. Expression of NEDD4 and its association with Itch may be promoted by T cell activation. Mice lacking NEDD4 exhibit inactivation of Itch and accumulation of Jun B, causing severe skin and lung inflammation and premature death.

REFERENCES

1. Harvey, K.F., et al. 2002. N4WBP5, a potential target for ubiquitination by the NEDD4 family of proteins, is a novel Golgi-associated protein. *J. Biol. Chem.* 277: 9307-9317.
2. Donnison, M., et al. 2004. Isolation of genes associated with developmentally competent bovine oocytes and quantitation of their levels during development. *Biol. Reprod.* 71: 1813-1821.
3. Shearwin-Whyatt, L.M., et al. 2004. N4WBP5A (NDFIP1), a NEDD4-interacting protein, localizes to multivesicular bodies and the Golgi, and has a potential role in protein trafficking. *J. Cell Sci.* 117: 3679-3689.
4. Bennett, C.L., et al. 2005. Genetic heterogeneity for autosomal recessive pyridoxine-dependent seizures. *Neurogenetics* 6: 143-149.
5. Oliver, P.M., et al. 2006. NDFIP1 protein promotes the function of itch ubiquitin ligase to prevent T cell activation and T helper 2 cell-mediated inflammation. *Immunity* 25: 929-940.
6. Zhang, Y., et al. 2006. Differential expression profiling between the relative normal and dystrophic muscle tissues from the same LGMD patient. *J. Transl. Med.* 4: 53.

CHROMOSOMAL LOCATION

Genetic locus: Nedd4 (rat) mapping to 8q24.

PRODUCT

NEDD4 siRNA (r) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NEDD4 shRNA Plasmid (r): sc-270215-SH and NEDD4 shRNA (r) Lentiviral Particles: sc-270215-V as alternate gene silencing products.

For independent verification of NEDD4 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270215A, sc-270215B and sc-270215C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NEDD4 siRNA (r) is recommended for the inhibition of NEDD4 expression in rat 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 μ M in 66 μ l. 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

NEDD4 (F-11): sc-518160 is recommended as a control antibody for monitoring of NEDD4 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 NEDD4 gene expression knockdown using RT-PCR Primer: NEDD4 (r)-PR: sc-270215-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.