SANTA CRUZ BIOTECHNOLOGY, INC.

Ndfip1 siRNA (m): sc-75887

BACKGROUND

Nedd4 family interacting protein-1 (Ndfip1), also referred to as N4WBP5, is a member of a family of highly conserved proteins. It has three transmembrane domains in its carboxy terminal and two PY motifs in its amino terminal. Ndfip1 is a 221 amino acid, Golgi-associated protein that may play a role in Golgi structure and function. Ndfip1 binds the WW domains of a number of Nedd4 family members called HECT-type E3 ubiquitin ligases. Ndfip1 is strongly expressed in surviving neurons around a site of injury. This suggests that ubiquitination may be a possible survival strategy and Ndfip1 may act as a neuroprotective protein. Ndfip1 may also have an effect on the function of Itch, another E3 ubiquitin ligase. Expression of Ndfip1 and its association with Itch may be promoted by T cell activation. Mice lacking Ndfip1 exhibit inactivation of Itch and accumulation of JunB causing them to die prematurely with severe skin and lung inflammation.

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 (Ndfip2), 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.

CHROMOSOMAL LOCATION

Genetic locus: Ndfip1 (mouse) mapping to 18 B3.

PRODUCT

Ndfip1 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 Ndfip1 shRNA Plasmid (m): sc-75887-SH and Ndfip1 shRNA (m) Lentiviral Particles: sc-75887-V as alternate gene silencing products.

For independent verification of Ndfip1 (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-75887A, sc-75887B and sc-75887C.

PROTOCOLS

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

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

Ndfip1 siRNA (m) is recommended for the inhibition of Ndfip1 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

Ndfip1 (H-6): sc-515417 is recommended as a control antibody for monitoring of Ndfip1 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 Ndfip1 gene expression knockdown using RT-PCR Primer: Ndfip1 (m)-PR: sc-75887-PR (20 µl). 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.