SANTA CRUZ BIOTECHNOLOGY, INC.

Npl4 siRNA (m): sc-61228

BACKGROUND

The NPL4 gene encodes the 608-amino acid, endoplasmic reticulum and nuclear membrane protein Npl4 that forms a complex with p97/Cdc48p and Ufd1p. This complex plays a role in IP_3 receptor processing by recognizing ubiquitinated IP_3 receptors in the endoplasmic reticulum and delivering them to the proteasome for degradation. The Npl4 protein contains 8 potential N-myristoylation sites, 5 potential N-glycosylation sites, several phosphorylation sites, and a C-terminal zinc finger motif. This protein is 96% homologous to the rat Npl4 protein, 44% homologous to the *Caenorhabditis elegans* Npl4 protein, and 34% homologous to the *Saccharomyces cerevisiae* Npl4 protein. Mutations in the NPL4 gene cause defects in nuclear envelope morphology, nuclear protein import, and nuclear poly(A) RNA export.

REFERENCES

1. DeHoratius, C., et al. 1997. Nuclear transport defects and nuclear envelope alterations are associated with mutation of the *Saccharomyces cerevisiae* NPL4 gene. Mol. Biol. Cell 7: 1835-1855.
2. Fabre, E., et al. 1998. Yeast genetics to dissect the nuclear pore complex and nucleocytoplasmic trafficking. Annu. Rev. Genet. 31: 277-313.
3. Meyer, H.H., et al. 2000. A complex of mammalian ufd1 and npl4 links the AAA-ATPase, p97, to ubiquitin and nuclear transport pathways. EMBO J. 19: 2181-2192.
4. Botta, A., et al. 2001. Cloning and characterization of the gene encoding human NPL4, a protein interacting with the ubiquitin fusion-degradation protein (UFD1L). Gene 275: 39-46.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606590. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
6. Bruderer, RM. et al. 2004. The AAA ATPase p97/VCP interacts with its alternative co-factors, Ufd1-Npl4 and p47, through a common bipartite binding mechanism. J. Biol. Chem. 279: 49609-49616.

CHROMOSOMAL LOCATION

Genetic locus: Nploc4 (mouse) mapping to 11 E2.

PRODUCT

Npl4 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 Npl4 shRNA Plasmid (m): sc-61228-SH and Npl4 shRNA (m) Lentiviral Particles: sc-61228-V as alternate gene silencing products.

For independent verification of Npl4 (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-61228A, sc-61228B and sc-61228C.

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

Npl4 siRNA (m) is recommended for the inhibition of Npl4 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

Npl4 (D-1): sc-365796 is recommended as a control antibody for monitoring of Npl4 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 Npl4 gene expression knockdown using RT-PCR Primer: Npl4 (m)-PR: sc-61228-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.