SANTA CRUZ BIOTECHNOLOGY, INC.

NUB1 siRNA (m): sc-61238

BACKGROUND

NEDD8 is a ubiquitin-like protein that controls vital biological events through its conjugation to cullin proteins. NEDD8 ultimate buster-1 (NUB1), is a negative regulator of the NEDD8 system that recruits NEDD8 and its conjugates to the proteasome for degradation. It is, therefore, a cell growth regulator. The UBA domain of NUB1 is the specific acceptor for the linear ubiquitin precursor. NUB1 is composed of 601 amino acids. It is an interferon-inducible protein and predominantly localizes in the nucleus. NUB1 is specifically expressed in adult human testis, ovary, heart, and skeletal muscle tissues.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607981. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. van der Spuy, J., et al. 2003. The expression o rod and cone photoreceptor development. Invest. Ophthalmol. Vis. Sci. 44: 5396-5403.
3. Hipp, M.S., et al. 2004. NEDD8 ultimate buster-1L interacts with the ubiquitin-like protein FAT10 and accelerates its degradation. J. Biol. Chem. 279: 16503-16510.
4. Kanaya, K., et al. 2004. Abolished interaction of NUB1 with mutant AIPL1 involved in Leber congenital amaurosis. Biochem. Biophys. Res. Commun. 317: 768-773.
5. Tanaka, T., et al. 2004. NUB1-mediated targeting of the ubiquitin precursor UbC1 for its C-terminal hydrolysis. Eur. J. Biochem. 271: 972-982.
6. van der Spuy, J. and Cheetham, M.E. 2004. The Leber congenital amaurosis protein AIPL1 modulates the nuclear translocation of NUB1 and suppresses inclusion formation by NUB1 fragments. J. Biol. Chem. 279: 48038-48047.

CHROMOSOMAL LOCATION

Genetic locus: Nub1 (mouse) mapping to 5 A3.

PRODUCT

NUB1 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 NUB1 shRNA Plasmid (m): sc-61238-SH and NUB1 shRNA (m) Lentiviral Particles: sc-61238-V as alternate gene silencing products.

For independent verification of NUB1 (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-61238A, sc-61238B and sc-61238C.

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.

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

NUB1 siRNA (m) is recommended for the inhibition of NUB1 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

NUB1 (F-10): sc-377003 is recommended as a control antibody for monitoring of NUB1 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 NUB1 gene expression knockdown using RT-PCR Primer: NUB1 (m)-PR: sc-61238-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.