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NSF siRNA (r): sc-156016

BACKGROUND

Syntaxins were originally thought to be docking proteins, but have more recently been categorized as anchoring proteins that anchor themselves to the cytoplasmic surfaces of cellular membranes. Syntaxins have been shown to bind to various proteins involved in exocytosis, including VAMPs (vesicle-associated membrane proteins), NSF (N-ethylmaleimide-sensitive factor), SNAP 25 (synaptosomal-associated protein of 25 kDa), SNAPs (soluble NSF attachment proteins) and synaptotagmin. VAMPs, also designated synaptobrevins, including VAMP-1 and VAMP-2, and synaptotagmin, a protein that may function as an inhibitor of exocytosis, are vesicular proteins. SNAPs, including α - and γ -SNAP, are cytoplasmic proteins that bind to a membrane receptor complex composed of VAMP, SNAP 25 and syntaxin. SNAPs mediate the membrane binding of NSF, which is essential for membrane fusion reactions.

REFERENCES

1. Elferink, L.A., et al. 1993. A role for synaptotagmin (p65) in regulated exocytosis. *Cell* 72: 153-159.
2. Bennett, M.K., et al. 1993. The syntaxin family of vesicular transport receptors. *Cell* 74: 863-873.
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4. Hayashi, T., et al. 1994. Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. *EMBO J.* 13: 5051-5061.
5. Edelman, L., et al. 1995. Synaptobrevin binding to synaptophysin: a potential mechanism for controlling the exocytosis fusion machine. *EMBO J.* 14: 224-231.
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7. Lin, R.C. and Scheller, R.H. 1997. Structural organization of the synaptic exocytosis core complex. *Neuron* 19: 1087-1094.
8. Barnard, R.J., et al. 1997. Stimulation of NSF ATPase activity by α -SNAP is required for SNARE complex disassembly and exocytosis. *J. Cell Biol.* 139: 875-883.

CHROMOSOMAL LOCATION

Genetic locus: *Nsf* (rat) mapping to 10q32.1.

PRODUCT

NSF 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 NSF shRNA Plasmid (r): sc-156016-SH and NSF shRNA (r) Lentiviral Particles: sc-156016-V as alternate gene silencing products.

For independent verification of NSF (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-156016A, sc-156016B and sc-156016C.

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

NSF siRNA (r) is recommended for the inhibition of NSF 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

NSF (A-3): sc-515043 is recommended as a control antibody for monitoring of NSF 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 NSF gene expression knockdown using RT-PCR Primer: NSF (r)-PR: sc-156016-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.