

NSP3 siRNA (h): sc-44855

BACKGROUND

The Eph family of receptor tyrosine kinases has been implicated in many developmental patterning processes, including cell segregation, cell migration, and axon guidance. An intermediate that is involved in the signaling pathways of the Eph receptors is novel SH2-containing protein 3 (NSP3, also designated SH2 domain-containing Eph receptor-binding protein 1, SHEP1, Cas, or HEF1 associated signal transducer), expressed in both the embryonic and adult brain. NSP3 contains a Src homology 2 domain that binds to a conserved tyrosine-phosphorylated motif in the juxtamembrane region of the EphB2 receptor. NSP3 may itself be a target of EphB2 kinase activity since it becomes heavily tyrosine-phosphorylated in cells expressing activated EphB2. NSP3 directly links activated, tyrosine-phosphorylated Eph receptors to small Ras superfamily GTPases.

REFERENCES

1. Drescher, U., et al. 1995. *In vitro* guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. *Cell* 82: 359-370.
2. Drescher, U. 1997. The Eph family in the patterning of neural development. *Curr. Biol.* 7: R799-R807.
3. Pasquale, E.B. 1997. The Eph family of receptors. *Curr. Opin. Cell Biol.* 9: 608-615.
4. Wang, H.U., et al. 1997. Eph family transmembrane ligands can mediate repulsive guidance of trunk neural crest migration and motor axon outgrowth. *Neuron* 18: 383-396.
5. Meima, L., et al. 1997. Lerk2 (ephrin B1) is a collapsing factor for a subset of cortical growth cones and acts by a mechanism different from AL-1 (Ephrin A5). *Mol. Cell. Neurosci.* 9: 314-328.
6. Bruckner, K., et al. 1998. Signaling by Eph receptors and their Ephrin ligands. *Curr. Opin. Neurobiol.* 8: 375-382.
7. Flanagan, J.G. and Vanderhaeghen, P. 1998. The ephrins and Eph receptors in neural development. *Annu. Rev. Neurosci.* 21: 309-345.
8. Dodelet, V.C., et al. 1999. A novel signaling intermediate, SHEP1, directly couples Eph receptors to R-Ras and Rap1A. *J. Biol. Chem.* 274: 31941-31946.

CHROMOSOMAL LOCATION

Genetic locus: SH2D3C (human) mapping to 9q34.11.

PRODUCT

NSP3 siRNA (h) 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 NSP3 shRNA Plasmid (h): sc-44855-SH and NSP3 shRNA (h) Lentiviral Particles: sc-44855-V as alternate gene silencing products.

For independent verification of NSP3 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44855A, sc-44855B and sc-44855C.

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

NSP3 siRNA (h) is recommended for the inhibition of NSP3 expression in human 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

NSP3 (J-16.1): sc-100792 is recommended as a control antibody for monitoring of NSP3 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 NSP3 gene expression knockdown using RT-PCR Primer: NSP3 (h)-PR: sc-44855-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.