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Shank 3 siRNA (h): sc-42200

BACKGROUND

SH3 and multiple Ankyrin repeat domains 1-3 (Shank 1-3) of the Shank/ProSAP family are molecular scaffolds in the postsynaptic density (PSD). The PSD is an electron-dense structure underneath the postsynaptic plasma membrane of excitatory synapses that anchors and clusters glutamate receptors opposite to the presynaptic neurotransmitter release site. Shank proteins contain PDZ modular domains that coordinate the synaptic localization of ion channels, receptors, signaling enzymes and cell adhesion molecules. The PDZ domain mediates protein-protein interactions via the recognition of a conserved sequence motif at the C-terminus of their target protein(s). Shank recruits βPIX and PAK to spines to regulate postsynaptic structure and interacts with NMDA receptor and metabotropic glutamate receptor complexes. Transcript splice variation in the Shank family influences the spectrum of Shank-interacting proteins in the PSDs of adult and developing brain to ensure normal development.

REFERENCES

1. Lim, S., et al. 1999. Characterization of the Shank family of synaptic proteins. Multiple genes, alternative splicing and differential expression in brain and development. *J. Biol. Chem.* 274: 29510-29518.
2. Sheng, M., et al. 2000. The Shank family of scaffold proteins. *J. Cell Sci.* 113: 1851-1856.
3. Tobaben, S., et al. 2000. The G protein-coupled receptor CL1 interacts directly with proteins of the Shank family. *J. Biol. Chem.* 275: 36204-36210.
4. Sala, C., et al. 2001. Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* 31: 115-130.
5. Boeckers, T.M., et al. 2002. ProSAP/Shank proteins—a family of higher order organizing molecules of the postsynaptic density with an emerging role in human neurological disease. *J. Neurochem.* 81: 903-910.
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CHROMOSOMAL LOCATION

Genetic locus: SHANK3 (human) mapping to 22q13.33.

PRODUCT

Shank 3 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Shank 3 shRNA Plasmid (h): sc-42200-SH and Shank 3 shRNA (h) Lentiviral Particles: sc-42200-V as alternate gene silencing products.

For independent verification of Shank 3 (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-42200A, sc-42200B and sc-42200C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Shank 3 siRNA (h) is recommended for the inhibition of Shank 3 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 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

Shank 3 (C-4): sc-377088 is recommended as a control antibody for monitoring of Shank 3 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 Shank 3 gene expression knockdown using RT-PCR Primer: Shank 3 (h)-PR: sc-42200-PR (20 μl, 397 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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