

NEEP21 siRNA (m): sc-62672

BACKGROUND

NEEP21 (neuron-enriched endosomal 21 kDa protein), also known as brain neuron cytoplasmic protein 1, NSG1 (neuron-specific proteins family member 1), P21 or D4S234E, is a single pass type II membrane protein belonging to the NSG family. It is highly expressed during neuronal maturation but its expression is downregulated in adult tissues. NEEP21 predominantly localizes to Rab 4-positive early endosomes in the somatodendritic neuronal compartment and is essential for proper receptor sorting and recycling in neurons. It associates with GRIP1 and GluR-2 and mediates the surface expression of GluR-2. When this interaction is interrupted, GluR-2 accumulates in early endosomes and leads to changes in evoked synaptic current properties. In addition, NEEP21 forms a complex with the SNARE protein, Syntaxin 13 (also known as Syntaxin 12), and participates in the recycling of transferrin receptors (TFRs) and NTR2 (neurotensin receptor 2).

REFERENCES

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2. Steiner, P., et al. 2002. Modulation of receptor cycling by neuron-enriched endosomal protein of 21 kD. *J. Cell Biol.* 157: 1197-1209.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607645. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Debaigt, C., et al. 2004. Crucial role of neuron-enriched endosomal protein of 21 kDa in sorting between degradation and recycling of internalized G protein-coupled receptors. *J. Biol. Chem.* 279: 35687-35691.
5. Steiner, P., et al. 2005. Interactions between NEEP21, GRIP1 and GluR2 regulate sorting and recycling of the glutamate receptor subunit GluR2. *EMBO J.* 24: 2873-2884.
6. Alberi, S., et al. 2005. The endosomal protein NEEP21 regulates AMPA receptor-mediated synaptic transmission and plasticity in the hippocampus. *Mol. Cell. Neurosci.* 29: 313-319.
7. Wang, Y. and Tang, B.L. 2006. SNAREs in neurons—beyond synaptic vesicle exocytosis (review). *Mol. Membr. Biol.* 23: 377-384.

CHROMOSOMAL LOCATION

Genetic locus: Nsg1 (mouse) mapping to 5 B3.

PRODUCT

NEEP21 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NEEP21 shRNA Plasmid (m): sc-62672-SH and NEEP21 shRNA (m) Lentiviral Particles: sc-62672-V as alternate gene silencing products.

For independent verification of NEEP21 (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-62672A, sc-62672B and sc-62672C.

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

NEEP21 siRNA (m) is recommended for the inhibition of NEEP21 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 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

NEEP21 (H-9): sc-390654 is recommended as a control antibody for monitoring of NEEP21 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 NEEP21 gene expression knockdown using RT-PCR Primer: NEEP21 (m)-PR: sc-62672-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.