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STEP siRNA (r): sc-270083

BACKGROUND

The brain-specific STEP (striatal enriched phosphatase) family of protein tyrosine phosphatases (PTPs) comprises both transmembrane and cytosolic protein members, which are the products of alternative splicing. STEP family members are expressed in the dopaminergic neurons of the CNS, with highest expression in the basal ganglia and related structures. The STEP protein regulates the N-methyl-D-aspartate receptor (NMDAR) complex; STEP depresses both NMDAR single-channel activity and synaptic currents. The membrane-associated STEP61 isoform localizes in the postsynaptic densities (PSDs) of striatal neurons. STEP61 contains a single tyrosine phosphatase domain, two proline-rich domains and two transmembrane domains. The STEP61 protein associates with the Src family kinase member Fyn when Fyn is phosphorylated at Tyr 420 and not Tyr 431. Upon association, STEP61 dephosphorylates Tyr 420 residue and may thus regulate Fyn activity in PSDs. Isolated from mouse brain, the STEP20 isoform lacks the conserved tyrosine phosphatase domain.

REFERENCES

- Lombroso, P.J., et al. 1993. A protein Tyrosine phosphatase expressed within dopaminergic neurons of the basal ganglia and related structures. *J. Neurosci.* 13: 3064-3074.
- Li, X., et al. 1995. Molecular cloning of the human homolog of a striatum-enriched phosphatase (STEP) gene and chromosomal mapping of the human and murine loci. *Genomics* 28: 442-449.
- Sharma, E., et al. 1995. Identification of two alternatively spliced transcripts of STEP: a subfamily of brain-enriched protein Tyrosine phosphatases. *Brain Res. Mol. Brain Res.* 32: 87-93.
- Bult, A., et al. 1997. STEP: a family of brain-enriched PTPs. Alternative splicing produces transmembrane, cytosolic and truncated isoforms. *Eur. J. Cell Biol.* 72: 337-344.
- Paul, S., et al. 2000. The dopamine/D1 receptor mediates the phosphorylation and inactivation of the protein Tyrosine phosphatase STEP via a PKA-dependent pathway. *J. Neurosci.* 20: 5630-5638.

CHROMOSOMAL LOCATION

Genetic locus: Ptpn5 (rat) mapping to 1q22.

PRODUCT

STEP 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 STEP shRNA Plasmid (r): sc-270083-SH and STEP shRNA (r) Lentiviral Particles: sc-270083-V as alternate gene silencing products.

For independent verification of STEP (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-270083A, sc-270083B and sc-270083C.

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 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

STEP siRNA (r) is recommended for the inhibition of STEP46 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

STEP (F-9): sc-514678 is recommended as a control antibody for monitoring of STEP 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 STEP46 gene expression knockdown using RT-PCR Primer: STEP (r)-PR: sc-270083-PR (20 μ l). 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.