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# STEP shRNA (m) Lentiviral Particles: sc-44480-V

## 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

1. 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.
2. 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.
3. 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.
4. 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.
5. 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 (mouse) mapping to 7 B4.

## PRODUCT

STEP shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10<sup>6</sup> infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see STEP siRNA (m): sc-44480 and STEP shRNA Plasmid (m): sc-44480-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

STEP shRNA (m) Lentiviral Particles is recommended for the inhibition of STEP expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10<sup>6</sup> infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

STEP (C-12): sc-22986 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor STEP gene expression knockdown using RT-PCR Primer: STEP (m)-PR: sc-44480-PR (20 µl, 583 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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