

SPR siRNA (h): sc-94595

BACKGROUND

SPR, also known as sepiapterin reductase, is a homodimeric cytoplasmic protein that belongs to the sepiapterin reductase family. SPR functions as an NADH-dependent aldo-keto reductase and specifically catalyzes the reduction of pteridine derivatives. In addition, SPR plays an important role in tetrahydrobiopterin (BH4) biosynthesis, catalyzing the final reduction step of the synthesis pathway. BH4 is an essential cofactor for the hydroxylation of the aromatic amino acids (tryptophan, tyrosine and phenylalanine) and is required for proper dopamine synthesis. Mutations in the gene encoding SPR can cause sepiapterin reductase deficiency, a monoamine neurotransmitter deficiency without hyperphenylalaninemia. Sepiapterin reductase deficiency interferes with BH4 synthesis, resulting in DOPA-responsive dystonia and a variety of other human diseases. In addition, SPR mRNA expression is increased in the brain of Parkinson's disease (PD) patients, suggesting that SPR may play a role in PD.

REFERENCES

1. Auerbach, G., Herrmann, A., Gülich, M., Fischer, M., Jacob, U., Bacher, A. and Huber, R. 1997. The 1.25 Å crystal structure of sepiapterin reductase reveals its binding mode to pterins and brain neurotransmitters. *EMBO J.* 16: 7219-7230.
2. Blau, N., Bonafé, L. and Thöny, B. 2001. Tetrahydrobiopterin deficiencies without hyperphenylalaninemia: diagnosis and genetics of dopa-responsive dystonia and sepiapterin reductase deficiency. *Mol. Genet. Metab.* 74: 172-185.
3. Ikemoto, K., Suzuki, T., Ichinose, H., Ohye, T., Nishimura, A., Nishi, K., Nagatsu, I. and Nagatsu, T. 2002. Localization of sepiapterin reductase in the human brain. *Brain Res.* 954: 237-246.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 182125. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Friedman, J., Hyland, K., Blau, N. and MacCollin, M. 2006. Dopa-responsive hypersomnia and mixed movement disorder due to sepiapterin reductase deficiency. *Neurology* 67: 2032-2035.

CHROMOSOMAL LOCATION

Genetic locus: SPR (human) mapping to 2p13.2.

PRODUCT

SPR 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 SPR shRNA Plasmid (h): sc-94595-SH and SPR shRNA (h) Lentiviral Particles: sc-94595-V as alternate gene silencing products.

For independent verification of SPR (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-94595A, sc-94595B and sc-94595C.

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

SPR siRNA (h) is recommended for the inhibition of SPR 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

SPR (A-11): sc-514777 is recommended as a control antibody for monitoring of SPR 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 SPR gene expression knockdown using RT-PCR Primer: SPR (h)-PR: sc-94595-PR (20 µl, 431 bp). 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.