SANTA CRUZ BIOTECHNOLOGY, INC.

TH siRNA (h): sc-36662

BACKGROUND

The enzyme tyrosine hydroxylase (TH), also designated tyrosine 3-monooxygenase (TY3H), catalyzes the conversion of tyrosine to L-DOPA, which is the rate limiting step in the biosynthesis of catecholamines such as dopamine, adrenalin and noradrenalin. TH is thought to play a role in the pathogenesis of Parkinson's disease, which is associated with reduced dopamine levels. Two transcription factor binding sites in the proximal region of the TH gene, the TPA-responsive element (TRE) and the c-AMP responsive element (CRE), have been implicated in the complex regulation of the TH gene. TH is also known to be upregulated by the glia maturation factor (GMF), a Cdc10/SWI6 motif-containing protein called V-1, and a variety of additional compounds.

REFERENCES

1. Stull, N.D., et al. 1996. Acidic fibroblast growth factor and catecholamines synergistically up-regulate tyrosine hydroxylase activity in developing and damaged dopamine neurons in culture. J. Neurochem. 67: 1519-1524.
2. Nagatsu, T., et al. 1998. Catecholamine synthesis and release. Overview. Adv. Pharmacol. 42: 1-14.
3. Haavik, J., et al. 1998. Tyrosine hydroxylase and Parkinson's disease. Mol. Neurobiol. 16: 285-309.
4. Trocme, C., et al. 1998. CRE and TRE sequences of the rat tyrosine hydroxylase promoter are required for TH basal expression in adult mice but not in the embryo. Eur. J. Neurosci. 10: 508-521.
5. Zaheer, A., et al. 1998. Overexpression of glia maturation factor (GMF) in PC12 pheochromocytoma cells activates p38 MAP kinase, MAPKAP kinase-2, and tyrosine hydroxylase. Biochem. Biophys. Res. Commun. 250: 278-282.
6. Yamakuni, T., et al. 1998. A novel protein containing Cdc10/SWI6 motifs regulates expression of mRNA encoding catecholamine biosynthesizing enzymes. J. Biol. Chem. 273: 27051-27054.
7. Boundy, V.A., et al. 1998. Regulation of tyrosine hydroxylase promoter activity by chronic morphine in TH9.0-LacZ transgenic mice. J. Neurosci. 18: 9989-9995.

CHROMOSOMAL LOCATION

Genetic locus: TH (human) mapping to 11p15.5.

PRODUCT

TH 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 TH shRNA Plasmid (h): sc-36662-SH and TH shRNA (h) Lentiviral Particles: sc-36662-V as alternate gene silencing products.

For independent verification of TH (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-36662A, sc-36662B and sc-36662C.

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

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

TH (F-11): sc-25269 is recommended as a control antibody for monitoring of TH 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 TH gene expression knockdown using RT-PCR Primer: TH (h)-PR: sc-36662-PR (20 µl, 579 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.