SANTA CRUZ BIOTECHNOLOGY, INC.

DBH siRNA (m): sc-35180

BACKGROUND

Dopamine β-hydroxylase (DBH) catalyzes the conversion of Dopamine to noradrenaline in the biosynthesis of catecholamines. DBH is selectively expressed in noradrenergic and adrenergic neurons, as well as in neuroendocrine cells, and it serves as a specific protein marker for noradrenergic processes. The active form of DBH is a homotetramer, which is found in the lumen of synaptic vesicles of corresponding nerve cells, where it localizes to both the membrane and cytosol. DBH is induced by nerve growth factor and Insulin growth factor-1 and is regulated by intracellular second messengers protein kinase A, cyclic AMP, diacyl glycerol and Ca^{2+}. Expression of DBH is transcriptionally mediated by Sp1, CREB and AP-1 proteins including c-Fos, c-Jun and JunD.

REFERENCES

1. Lamouroux, A., et al. 1987. The primary structure of human Dopamine-β-hydroxylase: insights into the relationship between the soluble and the membrane-bound forms of the enzyme. EMBO J. 6: 3931-3937.
2. Kobayashi, K., et al. 1989. Human Dopamine β-hydroxylase gene: two mRNA types having different 3'-terminal regions are produced through alternative polyadenylation. Nucleic Acids Res. 17: 1089-1102.
3. McMahon, A., et al. 1990. Rat Dopamine β-hydroxylase: molecular cloning and characterization of the cDNA and regulation of the mRNA by reserpine. J. Neurosci. Res. 25: 395-404.
4. Hwang, O., et al. 1995. Induction of gene expression of the catecholamine-synthesizing enzymes by Insulin-like growth factor-I. J. Neurochem. 65: 1988-1996.
5. Kim, H.S., et al. 1998. Noradrenergic-specific transcription of the Dopamine β-hydroxylase gene requires synergy of mulitple *cis*-acting elements including at least two Phox2a-binding sites. J. Neurosci. 18: 8247-8260.
6. Swanson, D.J., et al. 1998. AP1 proteins mediate the cAMP response of the Dopamine β-hydroylase gene. J. Biol. Chem. 273: 24065-24074.

CHROMOSOMAL LOCATION

Genetic locus: Dbh (mouse) mapping to 2 A3.

PRODUCT

DBH 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DBH shRNA Plasmid (m): sc-35180-SH and DBH shRNA (m) Lentiviral Particles: sc-35180-V as alternate gene silencing products.

For independent verification of DBH (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-35180A, sc-35180B and sc-35180C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

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

DBH (DBH 41): sc-47707 is recommended as a control antibody for monitoring of DBH 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-IgGλ BP-HRP: sc-516132 or m-IgGλ BP-HRP (Cruz Marker): sc-516132-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-IgGλ BP-FITC: sc-516185 or m-IgGλ BP-PE: sc-516186 (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 DBH gene expression knockdown using RT-PCR Primer: DBH (m)-PR: sc-35180-PR (20 µl). 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.