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▶ DAT siRNA (h): sc-41936

BACKGROUND

The members of the G protein-coupled receptor family are distinguished by their slow transmitting response to ligand binding. These seven transmembrane proteins include the adrenergic, serotonin and dopamine receptors. The effect of the signaling molecule can be excitatory or inhibitory, depending on the type of receptor to which it binds. β -adrenergic receptor bound to adrenaline activates adenylyl cyclase, while α_2 -adrenergic receptor bound to adrenaline inhibits adenylyl cyclase. The dopamine receptors are divided into two classes, D1 and D2, which differ in their functional characteristics in that D1 receptors stimulate adenylyl cyclase, while D2 receptors inhibit adenylyl cyclase activity. Five different subtypes of dopamine receptor have been described to date. D1DR and D5DR belong to the D1 subclass, while D2DR, D3DR and D4DR belong to the D2 subclass of dopamine receptors. The dopamine transporter, DAT, is a sodium and chloride-dependent dopamine transporter. DAT also can transport dopamine neurotoxins and has been implicated in the selective vulnerability of nigrostriatal dopaminergic neurons in major models of Parkinson's disease.

REFERENCES

1. Cotecchia, S., et al. 1990. Multiple second messenger pathways of α -adrenergic receptor subtypes expressed in eukaryotic cells. *J. Biol. Chem.* 265: 63-69.
2. Senogles, S.E. 1994. The D2 dopamine receptor isoforms signal through distinct $G_{\alpha i}$ proteins to inhibit adenylyl cyclase. A study with site-directed mutant $G_{\alpha i}$ proteins. *J. Biol. Chem.* 269: 23120-23127.
3. Barak, L.S., et al. 1995. The conserved seven-transmembrane sequence NP(X)2,3Y of the G protein-coupled receptor superfamily regulates multiple properties of the β_2 -adrenergic receptor. *Biochemistry* 34: 15407-15414.
4. Ng, G.Y., et al. 1995. Agonist-induced desensitization of dopamine D1 receptor-stimulated adenylyl cyclase activity is temporally and biochemically separated from D1 receptor internalization. *Proc. Natl. Acad. Sci. USA* 92: 10157-10161.
5. Ogawa, N. 1995. Molecular and chemical neuropharmacology of dopamine receptor subtypes. *Acta Med. Okayama* 49: 1-11.

CHROMOSOMAL LOCATION

Genetic locus: SLC6A3 (human) mapping to 5p15.33.

PRODUCT

DAT 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DAT shRNA Plasmid (h): sc-41936-SH and DAT shRNA (h) Lentiviral Particles: sc-41936-V as alternate gene silencing products.

For independent verification of DAT (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-41936A, sc-41936B and sc-41936C.

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

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

DAT (6-8D6): sc-32259 is recommended as a control antibody for monitoring of DAT 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DAT gene expression knockdown using RT-PCR Primer: DAT (h)-PR: sc-41936-PR (20 μ l). 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.