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LRRK2 siRNA (h): sc-45380

BACKGROUND

Parkinson's disease is a disorder of movement, cognition and emotion. It is characterized pathologically by neuronal degeneration with Lewy bodies, which are cytoplasmic inclusion bodies containing deposits of aggregated proteins. Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) cause autosomal-dominant Parkinsonism with clinical features of Parkinson's disease and with pleomorphic pathology including deposits of aggregated protein. The LRRK2 protein consists of multiple domains and belongs to the Roco family, a novel group of the Ras/GTPase superfamily. Besides the GTPase (Roc) domain, it contains a predicted kinase domain, with homology to MAP kinase kinase kinases. LRRK2 is localized in the cytoplasm and is associated with cellular membrane structures. The purified LRRK2 protein demonstrates autokinase activity.

REFERENCES

1. Zimprich, A., et al. 2004. Mutations in LRRK2 cause autosomal-dominant Parkinsonism with pleomorphic pathology. *Neuron* 44: 601-607.
2. Mata, I.F., et al. 2005. LRRK2 pathogenic substitutions in Parkinson's disease. *Neurogenetics* 6: 171-177.
3. Foroud, T., et al. 2005. LRRK2: both a cause and a risk factor for Parkinson disease? *Neurology* 65: 664-665.
4. Paisan-Ruiz, C., et al. 2005. LRRK2 gene in Parkinson disease: mutation analysis and case control association study. *Neurology* 65: 696-700.
5. Kachergus, J., et al. 2005. Identification of a novel LRRK2 mutation linked to autosomal dominant parkinsonism: evidence of a common founder across European populations. *Am. J. Hum. Genet.* 76: 672-680.
6. Bonifati, V., et al. 2005. Genetics of Parkinson's disease. *Minerva Med.* 96: 175-186.

CHROMOSOMAL LOCATION

Genetic locus: LRRK2 (human) mapping to 12q12.

PRODUCT

LRRK2 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 LRRK2 shRNA Plasmid (h): sc-45380-SH and LRRK2 shRNA (h) Lentiviral Particles: sc-45380-V as alternate gene silencing products.

For independent verification of LRRK2 (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-45380A, sc-45380B and sc-45380C.

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.

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

LRRK2 siRNA (h) is recommended for the inhibition of LRRK2 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

LRRK2 (133AT1218): sc-130159 is recommended as a control antibody for monitoring of LRRK2 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 LRRK2 gene expression knockdown using RT-PCR Primer: LRRK2 (h)-PR: sc-45380-PR (20 μ l, 491 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.