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LRRK2 shRNA (h) Lentiviral Particles: sc-45380-V

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

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CHROMOSOMAL LOCATION

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

PRODUCT

LRRK2 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see LRRK2 siRNA (h): sc-45380 and LRRK2 shRNA Plasmid (h): sc-45380-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

LRRK2 shRNA (h) Lentiviral Particles is recommended for the inhibition of LRRK2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

LRRK2 (M-300): sc-66954 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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