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PINK1 shRNA (m) Lentiviral Particles: sc-44599-V

BACKGROUND

A member of the serine/threonine protein kinase family, PTEN-induced putative kinase 1 (PINK1) is a tumor suppressor. PINK1 is primarily located in mitochondria and is ubiquitously expressed in testis, skeletal muscle and heart tissue. It can also be detected at lower levels in pancreas, ovary, brain, placenta, kidney, liver, prostate and small intestine. During cellular stress PINK1 protects against mitochondrial dysfunction by inducing phosphorylation mitochondrial proteins. PINK1 mutations may give rise to different autophosphorylation activity. Mutations in the PINK1 gene (PINK1) are associated with early onset Parkinson's disease, a recessive neurodegenerative disorder characterized by resting tremor, muscular rigidity, bradykinesia and postural instability. Parkinson's disease generally involves the presence of intraneuronal accumulations of aggregated proteins (Lewy bodies) in brain neurons.

REFERENCES

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2. Rogava, E., et al. 2004. Analysis of the PINK1 gene in a large cohort of cases with Parkinson disease. *Arch. Neurol.* 61: 1898-1904.
3. Healy, D.G., et al. 2004. The gene responsible for PARK6 Parkinson's disease, PINK1, does not influence common forms of parkinsonism. *Ann. Neurol.* 56: 329-335.
4. Hatano, Y., et al. 2004. Novel PINK1 mutations in early-onset parkinsonism. *Ann. Neurol.* 56: 424-427.
5. Valente, E.M., et al. 2004. Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* 304: 1158-1160.
6. Silvestri, L., et al. 2005. Mitochondrial import and enzymatic activity of PINK1 mutants associated to recessive parkinsonism. *Hum. Mol. Genet.* 14: 3477-3492.
7. Fung, H.C., et al. 2006. Analysis of the PINK1 gene in a cohort of patients with sporadic early-onset parkinsonism in Taiwan. *Neurosci. Lett.* 394: 33-36.

CHROMOSOMAL LOCATION

Genetic locus: Pink1 (mouse) mapping to 4 D3.

PRODUCT

PINK1 shRNA (m) 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 PINK1 siRNA (m): sc-44599 and PINK1 shRNA Plasmid (m): sc-44599-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

PINK1 shRNA (m) Lentiviral Particles is recommended for the inhibition of PINK1 expression in mouse 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

PINK1 (H-300): sc-33796 is recommended as a control antibody for monitoring of PINK1 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 PINK1 gene expression knockdown using RT-PCR Primer: PINK1 (m)-PR: sc-44599-PR (20 μ l, 564 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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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