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α/β -synuclein shRNA (h) Lentiviral Particles: sc-43589-V

BACKGROUND

The synucleins, including α -synuclein (also designated NACP for nonamyloid component precursor), β -synuclein (also designated PNP 14 for phospho-neuroprotein 14) and γ -synuclein (also designated persyn or BCSG1 for breast cancer-specific gene 1) are presynaptic proteins abundant in neurons. Synucleins are predominantly expressed in the brain and are speculated to be involved in synaptic regulation and neuronal plasticity. α -Synuclein, identified as a component of Alzheimer's disease amyloid plaques, is localized to neuronal cell bodies and synapses. Coordinate expression of α -synuclein and β -synuclein may be important during hematopoietic cell differentiation. A mutant form of α -synuclein is found in patients with early onset Parkinson's disease. γ -Synuclein is associated with axonal pathology in Parkinson's disease.

REFERENCES

1. Ueda, K., et al. 1993. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer's disease. Proc. Natl. Acad. Sci. USA 90: 11282-11286.
2. Jakes, R., et al. 1994. Identification of two distinct synucleins from human brain. FEBS Letts. 345: 27-32.
3. Iwai, A., et al. 1995. The precursor protein of non-A β component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. Neuron 14: 467-475.
4. Polymeropoulos, M.H., et al. 1997. Mutation in the α -synuclein gene identified in families with Parkinson's disease. Science 276: 2045-2047.

CHROMOSOMAL LOCATION

Genetic locus: SNCA (human) mapping to 4q22.1, SNCB (human) mapping to 5q35.2.

PRODUCT

α/β -synuclein 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 α/β -synuclein siRNA (h): sc-43589 and α/β -synuclein shRNA Plasmid (h): sc-43589-SH as alternate gene silencing products.

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.

APPLICATIONS

α/β -synuclein shRNA (h) Lentiviral Particles is recommended for the inhibition of α -synuclein and β -synuclein 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

α/β -synuclein (Syn 202): sc-32281 is recommended as a control antibody for monitoring of α/β -synuclein 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor α -synuclein and β -synuclein gene expression knockdown using RT-PCR Primer: α/β -synuclein (h)-PR: sc-43589-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

PROTOCOLS

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