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PDE4D shRNA (h) Lentiviral Particles: sc-44004-V

BACKGROUND

Phosphodiesterases (PDE) hydrolyze cAMP to 5' AMP and thus play a critical role in the regulation of intracellular cAMP. Division of the PDE superfamily by sequence homology and enzymatic properties yields 11 PDE families. A unique upstream conserved region (UCR) characterizes the PDE4 family. Four separate genes (A-D) encode the PDE4 enzymes, and alternative splicing generates short or long isoforms of each gene. Long PDE4 isoforms contain both UCR1 and UCR2 while short PDE4 isoforms possess only UCR2. Both UCR domains are necessary for dimerization of PDE4 isoforms. The human PDE4D gene maps to chromosome 5q11.2. The splice variants include isoforms PDE4D1-6.

REFERENCES

1. Bolger, G., et al. 1993. A family of human phosphodiesterases homologous to the dunce learning and memory gene product of *Drosophila melanogaster* are potential targets for antidepressant drugs. *Mol. Cell Biol.* 13: 6558-6571.
2. Milatovich, A., et al. 1994. Chromosome localizations of genes for five cAMP-specific phosphodiesterases in man and mouse. *Somat. Cell Mol. Genet.* 20: 75-86.
3. Francis, S.H., et al. 2001. Cyclic nucleotide phosphodiesterases: relating structure and function. *Prog. Nucleic Acid Res. Mol. Biol.* 65: 1-52.
4. Richter, W., et al. 2002. Dimerization of the type 4 cAMP-specific phosphodiesterases is mediated by the upstream conserved regions (UCRs). *J. Biol. Chem.* 277: 40212-40221.

CHROMOSOMAL LOCATION

Genetic locus: PDE4D (human) mapping to 5q11.2.

PRODUCT

PDE4D 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 PDE4D siRNA (h): sc-44004 and PDE4D shRNA Plasmid (h): sc-44004-SH as alternate gene silencing products.

APPLICATIONS

PDE4D shRNA (h) Lentiviral Particles is recommended for the inhibition of PDE4D isoform 1 expression in human 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.

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

PDE4D (K-16): sc-25100 is recommended as a control antibody for monitoring of PDE4D isoform 1 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PDE4D isoform 1 gene expression knockdown using RT-PCR Primer: PDE4D (h)-PR: sc-44004-PR (20 μ l, 509 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.