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- Expressversand

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# PDE5A shRNA (h) Lentiviral Particles: sc-44918-V

## BACKGROUND

Phosphodiesterases (PDE, also designated cyclic nucleotide phosphodiesterase) are important for the downregulation of the intracellular level of the second messenger cyclic adenosine monophosphate (cAMP) by hydrolyzing cAMP to 5'AMP. The PDE family contains proteins that serve tissue-specific roles in the regulation of lipolysis, glycogenolysis, myocardial contractility and smooth muscle relaxation. PDE5A, also designated cGMP-binding cGMP-specific phosphodiesterase or CGB-PDE, regulates the intracellular concentration of cyclic nucleotides and thereby is important in signal transduction. PDE5A catalyzes the hydrolysis of cGMP to 5'GMP and the protein is expressed in heart, placenta, aortic smooth muscle cells, skeletal muscle and pancreas.

## REFERENCES

- Cheung, P.P., Yu, L., Zhang, H. and Colman, R.W. 1998. Partial characterization of the active site human platelet cAMP phosphodiesterase, PDE3A, by site-directed mutagenesis. *Arch. Biochem. Biophys.* 360: 99-104.
- Gantner, F., Gotz, C., Gekeler, V., Schudt, C., Wendel, A. and Hatzelmann, A. 1998. Phosphodiesterase profile of human B lymphocytes from normal and atopic donors and the effects of PDE inhibition on B cell proliferation. *Br. J. Pharmacol.* 123: 1031-1038.
- Fisher, D.A., Smith, J.F., Pillar, J.S., St. Denis, S.H. and Cheng, J.B. 1998. Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. *Biochem. Biophys. Res. Commun.* 246: 570-577.
- Hetman, J.M., Soderling, S.H., Glavas, N.A. and Beavo, J.A. 2000. Cloning and characterization of PDE7B, a cAMP-specific phosphodiesterase. *Proc. Natl. Acad. Sci. USA* 97: 472-476.
- SWISS-PROT/TrEMBL (O60930). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

## CHROMOSOMAL LOCATION

Genetic locus: PDE5A (human) mapping to 4q27.

## PRODUCT

PDE5A 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 PDE5A siRNA (h): sc-44918 and PDE5A shRNA Plasmid (h): sc-44918-SH as alternate gene silencing products.

## STORAGE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

PDE5A shRNA (h) Lentiviral Particles is recommended for the inhibition of PDE5A expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

PDE5A (D-3): sc-398747 is recommended as a control antibody for monitoring of PDE5A 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 PDE5A gene expression knockdown using RT-PCR Primer: PDE5A (h)-PR: sc-44918-PR (20  $\mu$ l). 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.