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PDE5A siRNA (m): sc-44919

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 (mouse) mapping to 3 G1.

PRODUCT

PDE5A siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PDE5A shRNA Plasmid (m): sc-44919-SH and PDE5A shRNA (m) Lentiviral Particles: sc-44919-V as alternate gene silencing products.

For independent verification of PDE5A (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44919A, sc-44919B and sc-44919C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PDE5A siRNA (m) is recommended for the inhibition of PDE5A expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PDE5A gene expression knockdown using RT-PCR Primer: PDE5A (m)-PR: sc-44919-PR (20 μ l, 538 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.