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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

PDE8A siRNA (h): sc-41617

BACKGROUND

Phosphodiesterases (PDEs) are important for the downregulation of the intracellular level of the second messenger cyclic adenosine monophosphate (cAMP) by hydrolyzing cAMP to 5'AMP. Human cyclic GMP-stimulated 3',5'-cyclic nucleotide phosphodiesterase (PDE2A) is expressed in cerebellum, neocortex, heart, kidney, placenta, lung, pulmonary artery, skeletal muscle and pancreas. PDE2A expression is detected in venous and capillary endothelial cells in cardiac and renal tissue. PDE8A is a high affinity cAMP-specific protein that is expressed in a wide variety of tissues including testis, ovary, small intestine, and colon. PDE8B is expressed specifically and abundantly in the thyroid gland and shares 65% sequence identity (83% similarity) with PDE8A.

REFERENCES

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2. 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.
3. 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.
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5. Hayashi, M., Matsushima, K., Ohashi, H., Tsunoda, H., Murase, S., Kawarada, Y. and Tanaka, T. 1998. Molecular cloning and characterization of human PDE8B, a novel thyroid-specific isozyme of 3',5'-cyclic nucleotide phosphodiesterase. *Biochem. Biophys. Res. Commun.* 250: 751-756.
6. Sadhu, K., Hensley, K., Florio, V.A. and Wolda, S.L. 1999. Differential expression of the cyclic GMP-stimulated phosphodiesterase PDE2A in human venous and capillary endothelial cells. *J. Histochem. Cytochem.* 47: 895-906.

CHROMOSOMAL LOCATION

Genetic locus: PDE8A (human) mapping to 15q25.3.

PRODUCT

PDE8A siRNA (h) 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 PDE8A shRNA Plasmid (h): sc-41617-SH and PDE8A shRNA (h) Lentiviral Particles: sc-41617-V as alternate gene silencing products.

For independent verification of PDE8A (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41617A, sc-41617B and sc-41617C.

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

PDE8A siRNA (h) is recommended for the inhibition of PDE8A expression in human 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

PDE8A (1H6): sc-293342 is recommended as a control antibody for monitoring of PDE8A 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 PDE8A gene expression knockdown using RT-PCR Primer: PDE8A (h)-PR: sc-41617-PR (20 μ l, 455 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.