SANTA CRUZ BIOTECHNOLOGY, INC.

PDE1A siRNA (m): sc-62764

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 PDE1 family are calmodulin-dependent (CaM-PDE) proteins that undergo stimulation through a calcium-calmodulin complex. The activation of PDE1A requires a sustained influx of Ca^{2+}. Excluding its two short unique regions, human PDE1A has a predicted amino acid sequence exhibiting 94% homology to PDE of cow origin. PDE1A is most highly expressed in the brain, heart, kidney and skeletal muscle.

REFERENCES

1. Clapham, J.C., et al. 2001. Cloning of dog heart PDE1A—a first detailed characterization at the molecular level in this species. Gene 268: 165-171.
2. Fidock, M., et al. 2002. Isolation and differential tissue distribution of two human cDNAs encoding PDE1 splice variants. Cell. Signal. 14: 53-60.
3. Lefievre, L., et al. 2002. Presence of cyclic nucleotide phosphodiesterases PDE1A, existing as a stable complex with calmodulin and PDE3A in human spermatozoa. Biol. Reprod. 67: 423-430.
4. Goraya, T.A., et al. 2004. Sustained entry of Ca^{2+} is required to activate Ca^{2+}-calmodulin-dependent phosphodiesterase 1A. J. Biol. Chem. 279: 40494-40504.
5. Ahlstrom, M., et al. 2005. Cyclic nucleotide phosphodiesterases (PDEs) in human osteoblastic cells; the effect of PDE inhibition on cAMP accumulation. Cell. Mol. Biol. Lett. 10: 305-319.
6. Vasta, V., et al. 2005. Identification of a new variant of PDE1A calmodulin-stimulated cyclic nucleotide phosphodiesterase expressed in mouse sperm. Biol. Reprod. 73: 598-609.
7. Evgenov, O.V., et al. 2006. Inhibition of phosphodiesterase 1 augments the pulmonary vasodilator response to inhaled nitric oxide in awake lambs with acute pulmonary hypertension. Am. J. Physiol. Lung Cell. Mol. Physiol. 290: L723-L729.

CHROMOSOMAL LOCATION

Genetic locus: Pde1a (mouse) mapping to 2 C3.

PRODUCT

PDE1A siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suit-able for 50-100 transfections. Also see PDE1A shRNA Plasmid (m): sc-62764-SH and PDE1A shRNA (m) Lentiviral Particles: sc-62764-V as alternate gene silencing products.

For independent verification of PDE1A (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-62764A, sc-62764B and sc-62764C.

RESEARCH USE

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

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PDE1A siRNA (m) is recommended for the inhibition of PDE1A 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 µM in 66 µl. 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

PDE1A (H-105): sc-50480 is recommended as a control antibody for monitoring of PDE1A 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PDE1A gene expression knockdown using RT-PCR Primer: PDE1A (m)-PR: sc-62764-PR (20 µl, 422 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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