SANTA CRUZ BIOTECHNOLOGY, INC.

PDE11A siRNA (h): sc-76095

BACKGROUND

cAMP and cGMP are 3',5'-cyclic nucleotides that function as second messengers in a wide variety of signaling pathways throughout the cell. The hydrolysis of cAMP and cGMP to their corresponding 5'-monophosphates (5'-AMP and 5'-GMP, respectively) is catalyzed by 3',5'-cyclic nucleotide phosphodiesterases (PDEs), which provide a means of downregulating cAMP and cGMP signaling. PDE11A (phosphodiesterase 11A), also known as PPNAD2, is a 934 amino acid cytoplasmic protein that belongs to the PDE family. Expressed as four isoforms that exhibit different tissue specificity, PDE11A contains two GAF domains through which it functions to catalyze the H_2O-dependent conversion of cAMP and cGMP to their hydrolyzed forms. PDE11A activity is pH-dependent and is regulated by the binding of free cGMP (which acts as an allosteric activator) to the GAF domains, an event that stimulates enzyme activity. Defects in the gene encoding PDE11A are the cause of primary pigmented nodular adrenocortical disease type 2 (PPNAD2), a bilateral adrenal defect that leads to endocrine-related Cushing syndrome.

REFERENCES

1. Hetman, J.M., et al. 2000. Cloning and characterization of two splice variants of human phosphodiesterase 11A. Proc. Natl. Acad. Sci. USA 97: 12891-12895.
2. Loughney, K., et al. 2005. 3',5'-cyclic nucleotide phosphodiesterase 11A: localization in human tissues. Int. J. Impot. Res. 17: 320-325.
3. D'Andrea, M.R., et al. 2005. Expression of PDE11A in normal and malignant human tissues. J. Histochem. Cytochem. 53: 895-903.
4. Horvath, A., et al. 2006. Adrenal hyperplasia and adenomas are associated with inhibition of phosphodiesterase 11A in carriers of PDE11A sequence variants that are frequent in the population. Cancer Res. 66: 11571-11575.
5. Gross-Langenhoff, M., et al. 2006. cAMP is a ligand for the tandem GAF domain of human phosphodiesterase 10 and cGMP for the tandem GAF domain of phosphodiesterase 11. J. Biol. Chem. 281: 2841-2846.
6. Horvath, A., et al. 2006. A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. Nat. Genet. 38: 794-800.

CHROMOSOMAL LOCATION

Genetic locus: PDE11A (human) mapping to 2q31.2.

PRODUCT

PDE11A 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PDE11A shRNA Plasmid (h): sc-76095-SH and PDE11A shRNA (h) Lentiviral Particles: sc-76095-V as alternate gene silencing products.

For independent verification of PDE11A (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-76095A, sc-76095B and sc-76095C.

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

PDE11A siRNA (h) is recommended for the inhibition of PDE11A 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 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

PDE11A (B-7): sc-365655 is recommended as a control antibody for monitoring of PDE11A 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 PDE11A gene expression knockdown using RT-PCR Primer: PDE11A (h)-PR: sc-76095-PR (20 µl). 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.