SANTA CRUZ BIOTECHNOLOGY, INC.

A cyclase VI siRNA (h): sc-40321

BACKGROUND

Adenylyl cyclases (A cyclases) function to convert ATP to cyclic AMP in response to activation by a variety of hormones, neurotransmitters and other regulatory molecules. Cyclic AMP, in turn, activates several other target molecules to control a broad range of diverse phenomena, such as metabolism, gene transcription and memory. A cyclases respond to receptor-initiated signals and are mediated by heterotrimeric G proteins which catalyze the exchange of GDP for GTP and activate A cyclase function. A cyclase VI, also known as ADCY6 (adenylate cyclase type 6), is a 1,168 amino acid A cyclase that localizes to the membrane and contains two guanylate cyclase domains. Using magnesium as a cofactor, A cyclase VI functions as a calcium-inhibitable A cyclase that catalyzes the conversion of ATP to 3',5'-cyclic AMP and diphosphate and plays a role in a variety of events throughout the body. Multiple isoforms of A cyclase VI exist due to alternative splicing events.

REFERENCES

1. Haber, N., et al. 1994. Chromosomal mapping of human adenylyl cyclase genes type III, type V and type VI. Hum. Genet. 94: 69-73.
2. Gaudin, C., et al. 1994. Mammalian adenylyl cyclase family members are randomly located on different chromosomes. Hum. Genet. 94: 527-529.
3. Harry, A., et al. 1997. Differential regulation of adenylyl cyclases by $G_{\alpha s}$. J. Biol. Chem. 272: 19017-19021.
4. Raimundo, S., et al. 1999. Cloning and sequence of partial cDNAs encoding the human type V and VI adenylyl cyclases and subsequent RNA-quantification in various tissues. Clin. Chim. Acta 285: 155-161.
5. Wicker, R., et al. 2000. Cloning and expression of human adenylyl cyclase type VI in normal thyroid tissues. Biochim. Biophys. Acta 1493: 279-283.
6. Côté, M., et al. 2001. Expression and regulation of adenylyl cyclase isoforms in the human adrenal gland. J. Clin. Endocrinol. Metab. 86: 4495-4503.
7. Ludwig, M.G. and Seuwen, K. 2002. Characterization of the human adenylyl cyclase gene family: cDNA, gene structure, and tissue distribution of the nine isoforms. J. Recept. Signal Transduct. Res. 22: 79-110.

CHROMOSOMAL LOCATION

Genetic locus: ADCY6 (human) mapping to 12q13.12.

PRODUCT

A cyclase VI 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 A cyclase VI shRNA Plasmid (h): sc-40321-SH and A cyclase VI shRNA (h) Lentiviral Particles: sc-40321-V as alternate gene silencing products.

For independent verification of A cyclase VI (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-40321A, sc-40321B and sc-40321C.

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

A cyclase VI siRNA (h) is recommended for the inhibition of A cyclase VI 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

A cyclase V/VI (B-6): sc-514785 is recommended as a control antibody for monitoring of A cyclase VI 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 A cyclase VI gene expression knockdown using RT-PCR Primer: A cyclase VI (h)-PR: sc-40321-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.