A cyclase VI siRNA (m): sc-40322



The Power to Question

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

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- 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.
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- Côté, M., et al. 2001. Expression and regulation of adenylyl cyclase isoforms in the human adrenal gland. J. Clin. Endocrinol. Metab. 86: 4495-4503.
- 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 (mouse) mapping to 15 F1.

PRODUCT

A cyclase VI 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 A cyclase VI shRNA Plasmid (m): sc-40322-SH and A cyclase VI shRNA (m) Lentiviral Particles: sc-40322-V as alternate gene silencing products.

For independent verification of A cyclase VI (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-40322A, sc-40322B and sc-40322C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

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

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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG 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 A cyclase VI gene expression knockdown using RT-PCR Primer: A cyclase VI (m)-PR: sc-40322-PR (20 μ I, 425 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.

Santa Cruz Biotechnology, Inc. 1.800.457.3801 831.457.3801 Fax 831.457.3801 Europe +00800 4573 8000 49 6221 4503 0 www.scbt.com