

A cyclase V siRNA (m): sc-40320

BACKGROUND

Adenylyl cyclases (A cyclases) function to convert ATP to cyclic AMP (cAMP) in response to activation by a variety of hormones, neurotransmitters and other regulatory molecules. cAMP, in turn, activates several other target molecules to control a broad range of diverse phenomena, including metabolism, gene transcription and memory. A cyclases respond to receptor-initiated signals, mediated by a variety of G_s and G_i heterotrimeric G proteins (such as $G_{\alpha s}$). The binding of an agonist to a $G_{\alpha s}$ -coupled receptor catalyzes the exchange of GDP (bound to $G_{\alpha s}$) for GTP, the dissociation of GTP- $G_{\alpha s}$ from $G_{\beta\gamma}$ and the subsequent $G_{\alpha s}$ -mediated activation of A cyclases. A cyclase V, also known as ADCY5, is a 1,261 amino acid Adenylyl cyclase that localizes to cellular membranes and contains two guanylate cyclase domains. Similar to other A cyclase proteins, A cyclase V uses magnesium as a cofactor to catalyze the conversion of ATP to cAMP.

REFERENCES

- Schlich, K., et al. 1997. Identification of an intramolecular interaction between small regions in type V adenylyl cyclase that influences stimulation of enzyme activity by $G_{\alpha s}$. *Proc. Natl. Acad. Sci. USA* 94: 9602-9607.
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- Dessauer, C.W., et al. 1998. Identification of a $G_{\alpha s}$ binding site on type V adenylyl cyclase. *J. Biol. Chem.* 273: 25831-25839.
- 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.
- Côte, M., et al. 2001. Expression and regulation of adenylyl cyclase isoforms in the human adrenal gland. *J. Clin. Endocrinol. Metab.* 86: 4495-4503.
- Patrizio, M., et al. 2001. Human immunodeficiency virus type 1 Tat protein decreases cyclic AMP synthesis in rat microglia cultures. *J. Neurochem.* 77: 399-407.
- Salim, S., et al. 2003. Identification of RGS2 and type V adenylyl cyclase interaction sites. *J. Biol. Chem.* 278: 15842-15849.

CHROMOSOMAL LOCATION

Genetic locus: *Adcy5* (mouse) mapping to 16 B3.

PRODUCT

A cyclase V 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 V shRNA Plasmid (m): sc-40320-SH and A cyclase V shRNA (m) Lentiviral Particles: sc-40320-V as alternate gene silencing products.

For independent verification of A cyclase V (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-40320A, sc-40320B and sc-40320C.

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

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

A cyclase V/VI (B-6): sc-514785 is recommended as a control antibody for monitoring of A cyclase V 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 A cyclase V gene expression knockdown using RT-PCR Primer: A cyclase V (m)-PR: sc-40320-PR (20 μ l, 524 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.