

A cyclase II siRNA (h): sc-40317

BACKGROUND

Adenylyl 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. Adenylyl cyclases respond to receptor-initiated signals, mediated by the G_s and G_i heterotrimeric G proteins. The binding of an agonist to a G_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 $G_{\alpha s}$ -mediated activation of adenylyl cyclase. Adenylyl cyclases of the type II family differ from other subforms in that they are conditionally stimulated by $G_{\alpha s/\beta\gamma}$ subunits and regulated by PKC-mediated C-terminal phosphorylation. Both short- and long-term activation of D(2L) dopamine receptors result in a marked degree of sensitization of AC I, AC II, AC V and AC IX, but not AC VIII. The effects on AC I, AC II and AC VIII is dependent upon the ability of these AC isoforms to synergistically respond to selective activators in the presence of activated $G_{\alpha s}$.

REFERENCES

1. Gilman, A.G. 1987. G proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56: 615-649.
2. Bourne, H.R., et al. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348: 125-132.
3. Tang, W.J., et al. 1992. Adenylyl cyclases. *Cell* 70: 869-872.
4. Taussig, R., et al. 1994. Distinct patterns of bidirectional regulation of mammalian adenylyl cyclases. *J. Biol. Chem.* 269: 6093-6100.
5. Jacobowitz, O. and Lyengar, R. 1994. Phorbol ester-induced stimulation and phosphorylation of adenylyl cyclase 2. *Proc. Natl. Acad. Sci. USA* 91: 10630-10634.
6. Bol, G.F., et al. 1997. Adenylyl cyclase type II is stimulated by PKC via C-terminal phosphorylation. *Biochim. Biophys. Acta* 1358: 307-313.
7. Cumbay, M.G. and Watts, V.J. 2001. Heterologous sensitization of recombinant adenylyl cyclases by activation of D_2 dopamine receptors. *J. Pharmacol. Exp. Ther.* 297: 1201-1209.

CHROMOSOMAL LOCATION

Genetic locus: ADCY2 (human) mapping to 5p15.31.

PRODUCT

A cyclase II 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see A cyclase II shRNA Plasmid (h): sc-40317-SH and A cyclase II shRNA (h) Lentiviral Particles: sc-40317-V as alternate gene silencing products.

For independent verification of A cyclase II (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-40317A, sc-40317B and sc-40317C.

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 II siRNA (h) is recommended for the inhibition of A cyclase II 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 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 II (F-7): sc-514938 is recommended as a control antibody for monitoring of A cyclase II 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 II gene expression knockdown using RT-PCR Primer: A cyclase II (h)-PR: sc-40317-PR (20 μ l). 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.