

A cyclase VIII siRNA (h): sc-40325

BACKGROUND

Adenylyl cyclases function to convert ATP to cyclic AMP in response to activation by a variety of hormones, neurotransmitters and other regulatory molecules. 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_{α_s}) for GTP, dissociation of $GTP-G_{\alpha_s}$ from $G_{\beta\gamma}$ and G_{α_s} -mediated activation of adenylyl cyclase. Adenylyl cyclase type VIII (AC VIII) is one of the three mammalian calcium-stimulated isoforms, each of which is expressed in a region-specific manner in the central nervous system. In addition to the high expression in the brain, A cyclase VIII is also expressed in the lung. Ca^{2+} /calmodulin-dependent A cyclase VIII immunoreactivity is increased in alcoholic corpus amygdaloideum and hippocampus, suggesting that adenylyl cyclase may play a role in the pathophysiology of alcoholism. A significant decrease in the level of A cyclase I and a tendency to decrease in the level of A cyclase VIII in Alzheimer's disease hippocampus, suggesting that A cyclase I and VIII may play an essential role in learning and memory. A cyclase VIII knock-out mice do not have normal increases in behavioral markers of anxiety; thus, A cyclase VIII may also function in the modulation of anxiety.

REFERENCES

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- Bourne, H.R., et al. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348: 125-132.
- Tang, W.J. and Gilman, A.G. 1992. Adenylyl cyclases. *Cell* 70: 869-872.
- Taussig, R., et al. 1994. Distinct patterns of bidirectional regulation of mammalian adenylyl cyclases. *J. Biol. Chem.* 269: 6093-6100.
- Muglia, L.M., et al. 1999. The 5'-flanking region of the mouse adenylyl cyclase type VIII gene imparts tissue-specific expression in transgenic mice. *J. Neurosci.* 19: 2051-2058.
- Yamamoto, M., et al. 2000. Hippocampal level of neural specific adenylyl cyclase type I is decreased in Alzheimer's disease. *Biochim. Biophys. Acta* 1535: 60-68.

CHROMOSOMAL LOCATION

Genetic locus: ADCY8 (human) mapping to 8q24.22.

PRODUCT

A cyclase VIII 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 VIII shRNA Plasmid (h): sc-40325-SH and A cyclase VIII shRNA (h) Lentiviral Particles: sc-40325-V as alternate gene silencing products.

For independent verification of A cyclase VIII (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-40325A, sc-40325B and sc-40325C.

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 VIII siRNA (h) is recommended for the inhibition of A cyclase VIII 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 VIII (B-6): sc-377323 is recommended as a control antibody for monitoring of A cyclase VIII 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 VIII gene expression knockdown using RT-PCR Primer: A cyclase VIII (h)-PR: sc-40325-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.