SANTA CRUZ BIOTECHNOLOGY, INC.

A cyclase X siRNA (h): sc-88117

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 X, also known as ADCY10 (adenylate cyclase 10), SAC, SACI, HCA2 or sacy, is a 1,610 amino acid soluble adenylyl cyclase that acts as a bicarbonate sensor throughout the body and plays an essential role in spermatogenesis. A member of the A cyclase family, A cyclase X exists as three alternatively spliced isoforms that localize to cytoplasm and cell membrane.

REFERENCES

1. Reed, B.Y., et al. 1999. Mapping a gene defect in absorptive hypercalciuria to chromosome 1q23.3-q24. J. Clin. Endocrinol. Metab. 84: 3907-3913.
2. Buck, J., et al. 1999. Cytosolic adenylyl cyclase defines a unique signaling molecule in mammals. Proc. Natl. Acad. Sci. USA 96: 79-84.
3. Sinclair, M.L., et al. 2000. Specific expression of soluble adenylyl cyclase in male germ cells. Mol. Reprod. Dev. 56: 6-11.
4. Chen, Y., et al. 2000. Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. Science 289: 625-628.
5. Reed, B.Y., et al. 2002. Identification and characterization of a gene with base substitutions associated with the absorptive hypercalciuria phenotype and low spinal bone density. J. Clin. Endocrinol. Metab. 87: 1476-1485.
6. Hess, K.C., et al. 2005. The "soluble" adenylyl cyclase in sperm mediates multiple signaling events required for fertilization. Dev. Cell 9: 249-259.

CHROMOSOMAL LOCATION

Genetic locus: ADCY10 (human) mapping to 1q24.2.

PRODUCT

A cyclase X 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 X shRNA Plasmid (h): sc-88117-SH and A cyclase X shRNA (h) Lentiviral Particles: sc-88117-V as alternate gene silencing products.

For independent verification of A cyclase X (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-88117A, sc-88117B and sc-88117C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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 X siRNA (h) is recommended for the inhibition of A cyclase X 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 X (B-1): sc-515097 is recommended as a control antibody for monitoring of A cyclase X 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 X gene expression knockdown using RT-PCR Primer: A cyclase X (h)-PR: sc-88117-PR (20 µl, 495 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.