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A cyclase V/VI shRNA (m) Lentiviral Particles: sc-43588-V

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 V (AC V) and VI (AC VI) have multiple messages. AC V and AC VI are highly expressed in heart. Unlike AC VI, AC V is expressed to a lesser extent in brain and is absent in a variety of other tissues. Both AC V and AC VI can be stimulated by NaF, guanosine 5'-[γ -thio]triphosphate and Forskolin but not by Ca^{2+} /calmodulin. Activation of the D2 dopaminergic and m4 muscarine receptors inhibit the activity of adenylyl cyclase isozymes I, V, VI and VIII, whereas type II, IV and VII are stimulated and type III is not affected.

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. Katsushika, S., et al. 1992. Cloning and characterization of a sixth adenylyl cyclase isoform: type V and VI constitute a subgroup within the mammalian adenylyl cyclase family. *Proc. Natl. Acad. Sci. USA* 89: 8774-8778.

CHROMOSOMAL LOCATION

Genetic locus: *Adcy5* (mouse) mapping to 16 B3, *Adcy6* (mouse) mapping to 15 F1.

PRODUCT

A cyclase V/VI shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see A cyclase V/VI siRNA (m): sc-43588 and A cyclase V/VI shRNA Plasmid (m): sc-43588-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

A cyclase V/VI shRNA (m) Lentiviral Particles is recommended for the inhibition of A cyclase V and A cyclase VI expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

A cyclase V/VI (C-17): sc-590 is recommended as a control antibody for monitoring of A cyclase V and 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor A cyclase V and A cyclase VI gene expression knockdown using RT-PCR Primer: A cyclase V/VI (m)-PR: sc-43588-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.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.