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G_{αq} shRNA (r) Lentiviral Particles: sc-45998-V

BACKGROUND

Heterotrimeric G proteins function to relay information from cell surface receptors to intracellular effectors. Each of a very broad range of receptors specifically detects an extracellular stimulus (a photon, pheromone, odorant, hormone or neurotransmitter) while the effectors (e.g. adenylyl cyclase), which act to generate one or more intracellular messengers, are less numerous. In mammals, G protein α , β and γ polypeptides are encoded by at least 16, 4 and 7 genes, respectively. Most interest in G proteins has been focused on their α subunits, since these proteins bind and hydrolyze GTP and most obviously regulate the activity of the best studied effectors. Four distinct classes of G _{α} subunits have been identified; these include G_s, G_i, G_q and G_{12/13}. The G_q class includes G_{α15}, G_{α14}, G_{α11} and G_{αq}, two of which, G_{α11} and G_{αq}, are abundant in brain and lung and present at lower levels in a variety of tissues.

REFERENCES

1. Strathmann, M., et al. 1990. G protein diversity: a distinct class of α subunits is present in vertebrates and invertebrates. *Proc. Natl. Acad. Sci. USA* 87: 9113-9117.
2. Simon, M.I., et al. 1991. Diversity of G proteins in signal transduction. *Science* 252: 802-808.
3. Cali, J.J., et al. 1992. Selective tissue distribution of G protein γ subunits, including a new form of the γ subunits identified by cDNA cloning. *J. Biol. Chem.* 267: 24023-24027.
4. von Weizsäcker, E., et al. 1992. Diversity among the β subunits of heterotrimeric GTP-binding proteins: characterization of a novel β subunit cDNA. *Biochem. Biophys. Res. Commun.* 183: 350-356.

CHROMOSOMAL LOCATION

Genetic locus: Gnaq (rat) mapping to 1q43.

PRODUCT

G_{αq} shRNA (r) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 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 x 10⁶ 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 G_{αq} siRNA (r): sc-45998 and G_{αq} shRNA Plasmid (r): sc-45998-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

G_{αq} shRNA (r) Lentiviral Particles is recommended for the inhibition of G_{αq} expression in rat cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ 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

G_{αq} (E-17): sc-393 is recommended as a control antibody for monitoring of G_{αq} 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 G_{αq} gene expression knockdown using RT-PCR Primer: G_{αq} (r)-PR: sc-45998-PR (20 μ l, 488 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.