

G_{αq} siRNA (h): sc-35429

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_{α12/13}. 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.

CHROMOSOMAL LOCATION

Genetic locus: GNAQ (human) mapping to 9q21.2.

PRODUCT

G_{αq} 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 G_{αq} shRNA Plasmid (h): sc-35429-SH and G_{αq} shRNA (h) Lentiviral Particles: sc-35429-V as alternate gene silencing products.

For independent verification of G_{αq} (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-35429A, sc-35429B and sc-35429C.

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

G_{αq} siRNA (h) is recommended for the inhibition of G_{αq} 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

G_{αq} (10): sc-136181 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 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 G_{αq} gene expression knockdown using RT-PCR Primer: G_{αq} (h)-PR: sc-35429-PR (20 μ l, 525 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Wang, P. and DeFea, K.A. 2006. Protease-activated receptor-2 simultaneously directs β -Arrestin-1-dependent inhibition and G_{αq}-dependent activation of phosphatidylinositol 3-kinase. *Biochemistry* 45: 9374-9385.
- Zoudilova, M., et al. 2007. β -Arrestin-dependent regulation of the cofilin pathway downstream of protease-activated receptor-2. *J. Biol. Chem.* 282: 20634-20646.
- Ho, A.L., et al. 2012. Impact of combined mTOR and MEK inhibition in uveal melanoma is driven by tumor genotype. *PLoS ONE* 7: e40439.
- Ambrosini, G., et al. 2013. Inhibition of mutant GNAQ signaling in uveal melanoma induces AMPK-dependent autophagic cell death. *Mol. Cancer Ther.* 12: 768-776.
- Turajlic, S., et al. 2014. Whole-genome sequencing reveals complex mechanisms of intrinsic resistance to BRAF inhibition. *Ann. Oncol.* 25: 959-967.
- Ambrosini, G., et al. 2014. Overexpression of DDX43 mediates MEK inhibitor resistance through Ras upregulation in uveal melanoma cells. *Mol. Cancer Ther.* 13: 2073-2080.
- Chen, J., et al. 2015. Platelet-activating factor receptor-mediated PI3K/AKT activation contributes to the malignant development of esophageal squamous cell carcinoma. *Oncogene* 34: 5114-5127.
- Herbst-Robinson, K.J., et al. 2015. Inflammatory eicosanoids increase amyloid precursor protein expression via activation of multiple neuronal receptors. *Sci. Rep.* 5: 18286.

RESEARCH USE

For research use only, not for use in diagnostic procedures.