

$G_{\alpha t1}$ siRNA (m): sc-45759

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 (i.e., 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_i class comprises all the known α subunits that are susceptible to pertussis toxin modifications, including $G_{\alpha i-1}$, $G_{\alpha i-2}$, $G_{\alpha i-3}$, $G_{\alpha o}$, $G_{\alpha t1}$, $G_{\alpha t2}$, $G_{\alpha z}$ and $G_{\alpha gust}$. In the well characterized visual system, photorhodopsin catalyzes the exchange of guanine nucleotides bound to the visual transducin G_{α} subunits ($G_{\alpha t1}$ in rod cells and $G_{\alpha t2}$ in cone cells).

REFERENCES

1. Jones, D.T. and Reed, R.R. 1987. Molecular cloning of five GTP-binding protein cDNA species from rat olfactory neuroepithelium. *J. Biol. Chem.* 262: 14241-14249.
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. McLaughlin, S.K., et al. 1992. Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature* 357: 563-569.
5. 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.
6. Conklin, B.R. and Bourne, H.R. 1993. Structural elements of G_{α} subunits that interact with $G_{\beta\gamma}$ receptors, and effectors. *Cell* 73: 631-641.

CHROMOSOMAL LOCATION

Genetic locus: Gnat1 (mouse) mapping to 9 F1.

PRODUCT

$G_{\alpha t1}$ siRNA (m) 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 $G_{\alpha t1}$ shRNA Plasmid (m): sc-45759-SH and $G_{\alpha t1}$ shRNA (m) Lentiviral Particles: sc-45759-V as alternate gene silencing products.

For independent verification of $G_{\alpha t1}$ (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45759A, sc-45759B and sc-45759C.

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

$G_{\alpha t1}$ siRNA (m) is recommended for the inhibition of $G_{\alpha t1}$ expression in mouse 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

$G_{\alpha t1}$ (C-9): sc-518139 is recommended as a control antibody for monitoring of $G_{\alpha t1}$ 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 MarkerTM 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 $G_{\alpha t1}$ gene expression knockdown using RT-PCR Primer: $G_{\alpha t1}$ (m)-PR: sc-45759-PR (20 μ l). 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.

PROTOCOLS

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