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G_α 12 siRNA (m): sc-41743

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., adeny 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_{αs}, G_{αi}, G_{αq} and G_{α12/13}. The two members of the fourth class of G_α subunit proteins, G_{α12} and G_{α13}, are insensitive to ADP-ribosylation by pertussis toxin, share 67% identity with each other and less than 45% identity with other G_α subunits and are widely expressed in a broad range of tissues.

REFERENCES

1. Strathmann, M., et al. 1989. Diversity of the G protein family: sequences from five additional α subunits in the mouse. *Proc. Natl. Acad. Sci. USA* 86: 7407-7409.
2. Strathmann, M.P., et al. 1991. G_{α12} and G_{α13} subunits define a fourth class of G protein α subunits. *Proc. Natl. Acad. Sci. USA* 88: 5582-5586.
3. Simon, M.I., et al. 1991. Diversity of G proteins in signal transduction. *Science* 252: 802-808.
4. 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.

CHROMOSOMAL LOCATION

Genetic locus: Gna12 (mouse) mapping to 5 G2.

PRODUCT

G_{α12} 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_{α12} shRNA Plasmid (m): sc-41743-SH and G_{α12} shRNA (m) Lentiviral Particles: sc-41743-V as alternate gene silencing products.

For independent verification of G_{α12} (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-41743A, sc-41743B and sc-41743C.

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_{α12} siRNA (m) is recommended for the inhibition of G_{α12} 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_{α12} (E-12): sc-515445 is recommended as a control antibody for monitoring of G_{α12} 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 Marker™ 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_{α12} gene expression knockdown using RT-PCR Primer: G_{α12} (m)-PR: sc-41743-PR (20 μ l, 458 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ki, S.H., et al. 2007. G_{α12} specifically regulates COX-2 induction by sphingosine 1-phosphate. Role for JNK-dependent ubiquitination and degradation of I κ B α . *J. Biol. Chem.* 282: 1938-1947.
2. Song, M.K., et al. 2018. G_{α12} regulates osteoclastogenesis by modulating NFATc1 expression. *J. Cell. Mol. Med.* 22: 849-860.

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.