SANTA CRUZ BIOTECHNOLOGY, INC.

G_βL siRNA (h): sc-75072

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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 (i.e. a photon, pheromone, odorant, hormone or neurotransmitter), while the effectors (e.g. adenyl 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. G_βL (G protein β subunit-like), also known as LST8, POP3 or WAT1, is a 326 amino acid protein that localizes to the cytoplasm and contains 7 WD repeats. Expressed in a variety of tissues with highest expression in heart, kidney and skeletal muscle, G_βL functions as a component of the TORC1 and TORC2 complex and plays an important role in cell growth response to environmental stimuli. Four isoforms of G_βL exist due to alternative splicing events.

REFERENCES

1. Rodgers, B.D., Levine, M.A., Bernier, M. and Montrose-Rafizadeh, C. 2001. Insulin regulation of a novel WD-40 repeat protein in adipocytes. J. Endocrinol. 168: 325-332.
2. Kim, D.H., Sarbassov, D.D., Ali, S.M., Latek, R.R., Guntur, K.V., Erdjument-Bromage, H., Tempst, P. and Sabatini, D.M. 2003. G_βL, a positive regulator of the Rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Mol. Cell 11: 895-904.
3. Oshiro, N., Yoshino, K., Hidayat, S., Tokunaga, C., Hara, K., Eguchi, S., Avruch, J. and Yonezawa, K. 2004. Dissociation of Raptor from mTOR is a mechanism of Rapamycin-induced inhibition of mTOR function. Genes Cells 9: 359-366.
4. Long, X., Lin, Y., Ortiz-Vega, S., Yonezawa, K. and Avruch, J. 2005. Rheb binds and regulates the mTOR kinase. Curr. Biol. 15: 702-713.
5. Sarbassov, D.D. and Sabatini, D.M. 2005. Redox regulation of the nutrient-sensitive raptor-mTOR pathway and complex. J. Biol. Chem. 280: 39505-39509.

CHROMOSOMAL LOCATION

Genetic locus: GBL (human) mapping to 16p13.3.

PRODUCT

G_βL 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see G_βL shRNA Plasmid (h): sc-75072-SH and G_βL shRNA (h) Lentiviral Particles: sc-75072-V as alternate gene silencing products.

For independent verification of G_βL (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-75072A, sc-75072B and sc-75072C.

PROTOCOLS

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

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

G_βL siRNA (h) is recommended for the inhibition of G_βL 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 µM in 66 µl. 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_βL (E-9): sc-514982 is recommended as a control antibody for monitoring of G_βL 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_βL gene expression knockdown using RT-PCR Primer: G_βL (h)-PR: sc-75072-PR (20 µl). 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.