SANTA CRUZ BIOTECHNOLOGY, INC.

GRK 6 siRNA (m): sc-35519

BACKGROUND

Heterotrimeric G protein-mediated signal transduction is a dynamically regulated process with the intensity of signal decreasing over time despite the continued presence of the agonist. This phenomenon, referred to as agonist-mediated desensitization, involves phosphorylation of the receptor by two classes of enzymes. The first class is comprised of the second messenger-regulated kinases, such as c-AMP dependent protein kinase A and protein kinase C. The second class includes the G protein-coupled receptor kinases (GRKs). At least seven members of the GRK family have been identified. These include rhodopsin kinase (GRK 1), two forms of β-adrenergic receptor kinase: GRK 2 (βARK, βARK1) and GRK 3 (βARK2), IT-11 (GRK 4), GRK 5, GRK 6 and GRK 7. Phosphorylation of receptors by GRKs appears to be strictly dependent on the receptor being in its agonist-activated state.

REFERENCES

1. Hausdorff, W.P., et al. 1990. Turning off the signal: desensitization of β-adrenergic receptor function. FASEB J. 4: 2881-2889.
2. Lorenz, W., et al. 1991. The receptor kinase family: primary structure of rhodopsin kinase reveals similarities to the β-adrenergic receptor kinase. Proc. Natl. Acad. Sci. USA 88: 8715-8719.
3. Benovic, J.L., et al. 1991. Cloning, expression and chromosomal localization of β-adrenergic receptor kinase 2. J. Biol. Chem. 266: 14939-14946.
4. Inglese, J., et al. 1993. Structure and mechanism of the G protein-coupled receptor kinases. J. Biol. Chem. 268: 23735-23738.
5. Liggett, S.B., et al. 1993. Structural basis for receptor subtype-specific regulation revealed by a chimeric β_3/β_2-adrenergic receptor. Proc. Natl. Acad. Sci. USA 90: 3665-3669.
6. Pei, G., et al. 1994. An approach to the study of G protein-coupled receptor kinases: an *in vitro*-purified membrane assay reveals differential receptor specificity and regulation by $G_{\beta\gamma}$ subunits. Proc. Natl. Acad. Sci. USA 91: 3633-3636.
7. Premont, R.T., et al. 1994. Identification, purification, and characterization of GRK 5, a member of the family of G protein-coupled receptor kinases. J. Biol. Chem. 269: 6832-6841.

CHROMOSOMAL LOCATION

Genetic locus: Grk6 (mouse) mapping to 13 B1.

PRODUCT

GRK 6 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GRK 6 shRNA Plasmid (m): sc-35519-SH and GRK 6 shRNA (m) Lentiviral Particles: sc-35519-V as alternate gene silencing products.

For independent verification of GRK 6 (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-35519A, sc-35519B and sc-35519C.

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

GRK 6 siRNA (m) is recommended for the inhibition of GRK 6 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 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

GRK 6 (XX-4): sc-100380 is recommended as a control antibody for monitoring of GRK 6 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 GRK 6 gene expression knockdown using RT-PCR Primer: GRK 6 (m)-PR: sc-35519-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.

PROTOCOLS

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