SANTA CRUZ BIOTECHNOLOGY, INC.

GGTase-Iβ siRNA (m): sc-40883

BACKGROUND

Eukaryotic cells contain three distinct prenyltransferases that catalyze the attachment of a thioether-linked 15-carbon farnesyl group or 20-carbon geranylgeranyl group to C-terminal cysteine residues. Geranylgeranyltransferase type I (GGTase-I, PGGTase-I) catalyzes the nucleophilic substitution reaction between geranylgeranyl diphosphate (GGPP) and a protein-derived thiol to form the thioether linkage. The candidate protein must contain a C-terminal CAAX motif in which "A" is an aliphatic amino acid and "X" is leucine. Geranylgeranylation is necessary for the TGFβ1 signaling pathway, which involves phosphatidylcholine-specific phospholipase and a protein kinase C. Human GGTase-I contains an α subunit and a β subunit. Geranylgeranyltransferase type II (GGTase-II) is a heterodimer that catalyzes the transfer of two 20-carbon geranylgeranyl groups from geranylgeranyl pyrophosphate onto C-terminal cysteine residues of Rab GTPases, which is required for the activity of Rab proteins. GGTase-II also contains an α subunit and a β subunit.

REFERENCES

1. Schafer, W.R., et al. 1992. Protein prenylation: genes, enzymes, targets, and functions. Annu. Rev. Genet. 26: 209-237.
2. van Bokhoven, H., et al. 1996. cDNA cloning and chromosomal localization of the genes encoding the α and β subunits of human Rab geranylgeranyl transferase: the 3' end of the α subunit gene overlaps with the transglutaminase 1 gene promoter. Genomics 38: 133-140.
3. Online Mendelian Inheritance in Man, OMIM™. 1997. Johns Hopkins University, Baltimore, MD. MIM Number: 602031. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Desnoyers, L., et al. 1998. Single prenyl-binding site on protein prenyl transferases. Proc. Natl. Acad. Sci. USA 95: 12266-12270.
5. Song, H.J., et al. 1998. Requirement for geranylgeranyl transferase I and acyl transferase in the TGFβ-stimulated pathway leading to elastin mRNA stabilization. Biochem. Biophys. Res. Commun. 252: 111-116.
6. Clausen, V.A., et al. 2001. Stereochemical analysis of the reaction catalyzed by human protein geranylgeranyl transferase. Biochemistry 40: 3920-3930.
7. Kalinin, A., et al. 2001. Expression of mammalian and its application for *in vitro* prenylation of Rab proteins. Protein Expr. Purif. 22: 84-91.

CHROMOSOMAL LOCATION

Genetic locus: Pggt1b (mouse) mapping to 18 C.

PRODUCT

GGTase-Iβ 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 GGTase-Iβ shRNA Plasmid (m): sc-40883-SH and GGTase-Iβ shRNA (m) Lentiviral Particles: sc-40883-V as alternate gene silencing products.

For independent verification of GGTase-Iβ (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-40883A, sc-40883B and sc-40883C.

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

GGTase-Iβ siRNA (m) is recommended for the inhibition of GGTase-Iβ 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

GGTase-Iβ (D-11): sc-376854 is recommended as a control antibody for monitoring of GGTase-Iβ 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 GGTase-Iβ gene expression knockdown using RT-PCR Primer: GGTase-Iβ (m)-PR: sc-40883-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.