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GGTase-II β shRNA (h) Lentiviral Particles: sc-45437-V

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 contains a C-terminal CAAX motif in which "A" is an aliphatic amino acid and "X" is leucine. Geranylgeranylation is necessary for the TGF β 1 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.

CHROMOSOMAL LOCATION

Genetic locus: RABGGTB (human) mapping to 1p31.1.

PRODUCT

GGTase-II β shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see GGTase-II β siRNA (h): sc-45437 and GGTase-II β shRNA Plasmid (h): sc-45437-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

GGTase-II β shRNA (h) Lentiviral Particles is recommended for the inhibition of GGTase-II β expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

GGTase-II β (B-8): sc-365926 is recommended as a control antibody for monitoring of GGTase-II β 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GGTase-II β gene expression knockdown using RT-PCR Primer: GGTase-II β (h)-PR: sc-45437-PR (20 μ l, 535 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.