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β-glucosidase shRNA (h) Lentiviral Particles: sc-44904-V

BACKGROUND

β-glucosidase is a predominantly liver enzyme which efficiently hydrolyzes β-D-glucoside and β-D-galactoside. Defects in β-glucosidase cause Gaucher disease, an inherited condition distinguished by the accumulation of glucosylceramide within the cells of the reticuloendothelial system. β-glucosidase is used in enzyme replacement treatment aimed at treating Gaucher disease. The absorption of dietary flavonoid glycosides in humans involves a critical deglycosylation step that is mediated by epithelial β-glucosidases.

REFERENCES

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- Zhao, L., et al. 2003. β-glucosylation as a part of self-resistance mechanism in methymycin/pikromycin producing strain *Streptomyces venezuelae*. *Biochemistry* 42: 14794-14804.
- Nemeth, K., et al. 2003. Deglycosylation by small intestinal epithelial cell β-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *Eur. J. Nutr.* 42: 29-42.
- Salvioli, R., et al. 2004. Glucosylceramidase mass and subcellular localization are modulated by cholesterol in Niemann-Pick disease type C. *J. Biol. Chem.* 279: 17674-17680.
- Paal, K., et al. 2004. *Paenibacillus* sp. TS12 glucosylceramidase: kinetic studies of a novel sub-family of family 3 glycosidases and identification of the catalytic residues. *Biochem. J.* 378: 141-149.

CHROMOSOMAL LOCATION

Genetic locus: GBA (human) mapping to 1q22.

PRODUCT

β-glucosidase 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 μl frozen stock containing 1.0 x 10⁶ 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 β-glucosidase siRNA (h): sc-44904 and β-glucosidase shRNA Plasmid (h): sc-44904-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

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0 x 10⁶ 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

β-glucosidase (C-2): sc-365745 is recommended as a control antibody for monitoring of β-glucosidase 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 β-glucosidase gene expression knockdown using RT-PCR Primer: β-glucosidase (h)-PR: sc-44904-PR (20 μl, 570 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.