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GH shRNA (m) Lentiviral Particles: sc-43804-V

BACKGROUND

Pituitary growth hormone (GH), also designated somatotropin, plays a crucial role in stimulating and controlling the growth, metabolism and differentiation of many mammalian cell types by modulating the synthesis of multiple mRNA species. These effects are mediated by the binding of GH to its membrane-bound receptor, GHR, and involve a phosphorylation cascade that results in the modulation of numerous signaling pathways. GH is secreted in a pulsatile pattern which is tightly controlled by the interplay of GH-releasing hormone (GHRH) and somatostatin (SRIF). GHRH and SRIF are the primary hypothalamic factors that determine GH secretion from the somatotroph and regulate GH synthesis and secretory reserve. GH output is also highly sensitive to feedback control by GH itself, as well as by Insulin-like growth factor I. GH is synthesized by acidophilic or somatotrophic cells of the anterior pituitary gland. Human growth hormone contains 191 amino acid residues with 2 disulfide bridges.

REFERENCES

1. Niall, H.D., et al. 1971. Sequence of pituitary and placental lactogenic and growth hormones: evolution from a primordial peptide by gene reduplication. *Proc. Natl. Acad. Sci. USA* 68: 866-869.
2. Harper, M.E., et al. 1982. Chromosomal localization of the human placental lactogen-growth hormone gene cluster to 17q22-q24. *Am. J. Hum. Genet.* 34: 227-234.
3. Jellinck, P.H., et al. 1985. Normal and recombinant human growth hormone administered by constant infusion feminize catechol estrogen formation by rat liver microsomes. *Endocrinology* 117: 2274-2278.
4. Campbell, R.M., et al. 1992. Evolution of the growth hormone-releasing factor (GRF) family of peptides. *Growth Regul.* 2: 175-191.

CHROMOSOMAL LOCATION

Genetic locus: Gh (mouse) mapping to 11 E1.

PRODUCT

GH shRNA (m) 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 GH siRNA (m): sc-43804 and GH shRNA Plasmid (m): sc-43804-SH as alternate gene silencing products.

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.

APPLICATIONS

GH shRNA (m) Lentiviral Particles is recommended for the inhibition of GH expression in mouse 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

GH (T-20): sc-10365 is recommended as a control antibody for monitoring of GH 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GH gene expression knockdown using RT-PCR Primer: GH (m)-PR: sc-43804-PR (20 μ l, 495 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.

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.