

ghrelin siRNA (h): sc-39517

BACKGROUND

Small synthetic molecules called growth-hormone secretagogues (GHSs) act through GSH-R to stimulate the release of GH from the pituitary. Ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R). Ghrelin and GHRH are involved in the regulation of GH release from the pituitary. GHRH exerts its action through high-affinity GHRH receptors (GHRH-R) present in the anterior pituitary. The acylated peptide of ghrelin specifically releases GH both *in vivo* and *in vitro* and is found in stomach tissue. GH 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. The gene encoding ghrelin maps to human chromosome 3p25.3.

REFERENCES

1. Campbell, R.M., et al. 1992. Evolution of the growth hormone-releasing factor (GRF) family of peptides. *Growth Regul.* 2: 175-191.
2. Mayo, K.E. 1992. Molecular cloning and expression of a pituitary-specific receptor for growth hormone-releasing hormone. *Mol. Endocrinol.* 6: 1734-1744.
3. Howard, A.D., et al. 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273: 974-977.
4. McKee, K.K., et al. 1997. Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. *Mol. Endocrinol.* 11: 415-423.
5. Bowers, C.Y. 1998. Growth hormone-releasing peptide (GHRP). *Cell. Mol. Life Sci.* 54: 1316-1329.
6. Kojima, M., et al. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656-660.
7. Date, Y., et al. 2001. ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochem. Biophys. Res. Commun.* 280: 904-907.
8. Toshinai, K., et al. 2001. Upregulation of Ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem. Biophys. Res. Commun.* 281: 1220-1225.

CHROMOSOMAL LOCATION

Genetic locus: GHRL (human) mapping to 3p25.3.

PRODUCT

ghrelin siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ghrelin shRNA Plasmid (h): sc-39517-SH and ghrelin shRNA (h) Lentiviral Particles: sc-39517-V as alternate gene silencing products.

For independent verification of ghrelin (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-39517A, sc-39517B and sc-39517C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ghrelin siRNA (h) is recommended for the inhibition of ghrelin expression in human 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 μ M in 66 μ l. 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

ghrelin (2F4): sc-293422 is recommended as a control antibody for monitoring of ghrelin 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 ghrelin gene expression knockdown using RT-PCR Primer: ghrelin (h)-PR: sc-39517-PR (20 μ l, 490 bp). 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.