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GCNF siRNA (m): sc-45761

BACKGROUND

Germ cell nuclear factor (GCNF) is an orphan member of the nuclear receptor gene superfamily that influences neurogenesis and germ cell development. GCNF can homodimerize and bind DNA. GCNF regulates paracrine interaction between the oocyte and somatic cells by regulating the expression of BMP-15 and GDF-9, to affect female fertility. GCNF is present in spermatocytes and round spermatids of adult male mouse testis; northern blot and ribonuclease protection assays have shown GCNF is predominant in the testis. The gene expresses three alternatively spliced transcript variants.

REFERENCES

- Chen, F., et al. 1994. Cloning of a novel orphan receptor (GCNF) expressed during germ cell development. *Mol. Endocrinol.* 8: 1434-1444.
- Kapelle, M., et al. 1997. cDNA cloning of two closely related forms of human germ cell nuclear factor (GCNF). *Biochim. Biophys. Acta* 1352: 13-17.
- Agoulnik, I.Y., et al. 1998. Cloning, expression analysis and chromosomal localization of the human nuclear receptor gene GCNF. *FEBS Lett.* 424: 73-78.
- Bauer, U.M., et al. 1998. The murine nuclear orphan receptor GCNF is expressed in the XY body of primary spermatocytes. *FEBS Lett.* 439: 208-214.
- Charles, J.P., et al. 1999. Characterization and DNA-binding properties of GRF, a novel monomeric binding orphan receptor related to GCNF and β FTZ-F1. *Eur. J. Biochem.* 266: 181-190.
- Schohl, A., et al. 2002. Oocytes and embryos of *Xenopus laevis* express two different isoforms of germ cell nuclear factor (GCNF, NR6A1). *Mech. Dev.* 118: 261-264.
- Lan, Z.J., et al. 2003. GCNF-dependent repression of BMP-15 and GDF-9 mediates gamete regulation of female fertility. *EMBO J.* 22: 4070-4081.

CHROMOSOMAL LOCATION

Genetic locus: Nr6a1 (mouse) mapping to 2 B.

PRODUCT

GCNF 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GCNF shRNA Plasmid (m): sc-45761-SH and GCNF shRNA (m) Lentiviral Particles: sc-45761-V as alternate gene silencing products.

For independent verification of GCNF (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-45761A, sc-45761B and sc-45761C.

PROTOCOLS

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

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

GCNF siRNA (m) is recommended for the inhibition of GCNF 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 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

GCNF (B-2): sc-271733 is recommended as a control antibody for monitoring of GCNF 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 GCNF gene expression knockdown using RT-PCR Primer: GCNF (m)-PR: sc-45761-PR (20 μ l, 515 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.