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GCNF shRNA (m) Lentiviral Particles: sc-45761-V

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

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- 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.
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- 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 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 GCNF siRNA (m): sc-45761 and GCNF shRNA Plasmid (m): sc-45761-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.

APPLICATIONS

GCNF shRNA (m) Lentiviral Particles is recommended for the inhibition of GCNF 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

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 (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 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 $55-60^{\circ}$ 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.

PROTOCOLS

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