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Eppin shRNA (h) Lentiviral Particles: sc-44415-V

BACKGROUND

Eppin, an epididymal protease inhibitor, belongs to the WFDC family and to the telomeric cluster. The gene encoding the protein is localized to chromosome 20q13.12 in centromeric and telomeric clusters. It expresses three mRNAs encoding two isoforms of a cystine-rich protein that contains Kunitz-type and WAP-type (four disulfide core) protease inhibitor consensus sequences. The mouse gene lies in a cluster of putative Eppin-like genes on mouse chromosome 2. Following ejaculation, Eppin is bound to semenogelin in seminal plasma and on human spermatozoa. This complex of Eppin and Semenogelin can provide antimicrobial activity for spermatozoa. It can also provide for the preparation and survival of spermatozoa for fertility in the female reproductive tract. Eppin, which is a secreted protein, is expressed in epididymis and testis.

REFERENCES

- Richardson, R.T., et al. 2001. Cloning and sequencing of human Eppin: a novel family of protease inhibitors expressed in the epididymis and testis. *Gene* 270: 93-102.
- Sivashanmugam, P., et al. 2003. Characterization of mouse Eppin and a gene cluster of similar protease inhibitors on mouse chromosome 2. *Gene* 312: 125-134.
- Karande, A., et al. 2004. Eppin: a candidate male contraceptive vaccine? *J. Biosci.* 29: 373-374.
- Yenugu, S., et al. 2004. Antimicrobial activity of human Eppin, an androgen-regulated, sperm-bound protein with a whey acidic protein motif. *Biol. Reprod.* 71: 1484-1490.
- Wang, Z., et al. 2005. Association of Eppin with semenogelin on human spermatozoa. *Biol. Reprod.* 72: 1064-1070.

CHROMOSOMAL LOCATION

Genetic locus: SPINLW1 (human) mapping to 20q13.12.

PRODUCT

Eppin 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 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 Eppin siRNA (h): sc-44415 and Eppin shRNA Plasmid (h): sc-44415-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

Eppin shRNA (h) Lentiviral Particles is recommended for the inhibition of Eppin expression in human 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

Eppin (I-12): sc-34604 is recommended as a control antibody for monitoring of Eppin 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 Eppin gene expression knockdown using RT-PCR Primer: Eppin (h)-PR: sc-44415-PR (20 μ l). 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.