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# Gelsolin shRNA (r) Lentiviral Particles: sc-45925-V

## BACKGROUND

Gelsolin (also known as brevin, Actin-depolymerizing factor or ADF), a protein of leukocytes, platelets and other cells, severs Actin filaments in the presence of submicromolar calcium, thereby isolating cytoplasmic Actin gels. A calcium-independent mechanism reverses the process. A Gelsolin variant with 23 more amino-terminal amino acids is a plasma component probably involved in the clearance of Actin, the most abundant human protein, from the circulation. It has been suggested that a single gene encodes both cell and plasma gelsolins. Gelsolin may be unique in that it is made for both secretion and intracytoplasmic location. Amino acid homology was identified between Gelsolin and the amyloid of the Finnish variety of amyloidosis. The amyloid in this disorder is antigenically and structurally related to Gelsolin. Gelsolin is the principal intracellular and extracellular Actin-severing protein. Gelsolin and Gc protein together constitute the extracellular Actin-scavenger system which prevents the toxic effects of Actin release into the extracellular space under circumstances of cell necrosis.

## REFERENCES

- Lind, S.E., et al. 1984. Human plasma Gelsolin binds to Fibronectin. *J. Biol. Chem.* 259: 13262-13266.
- Fernandes-Alnemri, T., et al. 1995. Mch3, a novel human apoptotic cysteine protease highly related to CPP32. *Cancer Res.* 55: 6045-6052.
- Takahashi, A., et al. 1996. Cleavage of Lamin A by Mch2 a but not CPP32: multiple interleukin-1  $\beta$ -converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. *Proc. Natl. Acad. Sci. USA* 93: 8395-8400.
- Rao, L., et al. 1996. Lamin proteolysis facilitates nuclear events during apoptosis. *J. Cell Biol.* 135: 1441-1455.
- Liu, X., et al. 1997. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger DNA fragmentation during apoptosis. *Cell* 89: 175-184.
- Salvesen, G.S., et al. 1997. Caspases: intracellular signaling by proteolysis. *Cell* 91: 443-446.

## CHROMOSOMAL LOCATION

Genetic locus: Gsn (rat) mapping to 3p11.

## PRODUCT

Gelsolin shRNA (r) 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 Gelsolin siRNA (r): sc-45925 and Gelsolin shRNA Plasmid (r): sc-45925-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

Gelsolin shRNA (r) Lentiviral Particles is recommended for the inhibition of Gelsolin expression in rat cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

Gelsolin (H-5): sc-398244 is recommended as a control antibody for monitoring of Gelsolin 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<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> 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<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Gelsolin gene expression knockdown using RT-PCR Primer: Gelsolin (r)-PR: sc-45925-PR (20  $\mu$ l). 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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