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# Geminin shRNA (h) Lentiviral Particles: sc-43800-V

## BACKGROUND

Geminin is a nuclear protein that regulates the initiation of DNA replication during the cell cycle. DNA replication requires the coordinated association of cdc6 and minichromosome maintenance (MCM) proteins with chromatin. Geminin blocks this assembly of the MCM into the prereplication complex and, in turn, prevents replication from occurring. Expression of geminin fluctuates throughout the cell cycle with geminin levels lowest at G<sub>1</sub>. Throughout S, G<sub>2</sub> and M phases, geminin levels are consistently elevated followed by a decrease during mitosis. The initiation of DNA replication is dependent on the degradation of geminin during mitosis and the absence of geminin throughout G<sub>1</sub> phase. Geminin degradation is mediated by the anaphase-promoting complex (APC), which specifically targets B-type cyclins and other proteins containing a destruction box motif for degradation by ubiquitin-mediated proteolysis.

## REFERENCES

1. Yu, H., et al. 1996. Identification of a novel ubiquitin-conjugating enzyme involved in mitotic cyclin degradation. *Curr. Biol.* 6: 455-466.
2. Rowles, A., et al. 1997. Chromatin proteins involved in the initiation of DNA replication. *Curr. Opin. Genet. Dev.* 7: 152-157.
3. Liang, C., et al. 1997. Persistent initiation of DNA replication and chromatin-bound MCM proteins during the cell cycle in cdc6 mutants. *Genes Dev.* 11: 3375-3386.
4. Page, A.M., et al. 1997. The anaphase promoting complex. *Cancer Surv.* 29: 133-150.
5. McGarry, T.J., et al. 1998. Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* 93: 1043-1053.
6. Kroll, K.L., et al. 1998. Geminin, a neuralizing molecule that demarcates the future neural plate at the onset of gastrulation. *Development* 125: 3247-3258.

## CHROMOSOMAL LOCATION

Genetic locus: GMNN (human) mapping to 6p22.3.

## PRODUCT

Geminin shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10<sup>6</sup> 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 Geminin siRNA (h): sc-43800 and Geminin shRNA Plasmid (h): sc-43800-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

Geminin shRNA (h) Lentiviral Particles is recommended for the inhibition of Geminin expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10<sup>6</sup> 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

Geminin (F-7): sc-74456 is recommended as a control antibody for monitoring of Geminin 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 Geminin gene expression knockdown using RT-PCR Primer: Geminin (h)-PR: sc-43800-PR (20 µl, 528 bp). 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.

## 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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