SANTA CRUZ BIOTECHNOLOGY, INC.

Geminin siRNA (m): sc-108025

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_1. Throughout S, G_2 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_1 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. 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.
6. McGarry, T.J., et al. 1998. Geminin, an inhibitor of DNA replication, is degraded during mitosis. Cell 93: 1043-1053.

CHROMOSOMAL LOCATION

Genetic locus: Gmnn (mouse) mapping to 13 A3.1.

PRODUCT

Geminin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Geminin shRNA Plasmid (m): sc-108025-SH and Geminin shRNA (m) Lentiviral Particles: sc-108025-V as alternate gene silencing products.

For independent verification of Geminin (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-108025A, sc-108025B and sc-108025C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Geminin siRNA (m) is recommended for the inhibition of Geminin 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 µM in 66 µl. 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

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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Geminin gene expression knockdown using RT-PCR Primer: Geminin (m)-PR: sc-108025-PR (20 µl). 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.