SANTA CRUZ BIOTECHNOLOGY, INC.

Cdt1 siRNA (m): sc-142240

BACKGROUND

Human Cdt1 is a nuclear localizing replication initiation factor that is expressed only during the G_1 and S phases of the cell cycle. In conjunction with Cdc18, Cdt1 is required to load the MCM protein Cdc21 onto chromatin at the end of mitosis which is necessary to initiate DNA replication. After S-phase onset, Cdt1 protein levels decrease and are barely detectable in cells in early S-phase or G_2. However, Cdt1 mRNA is expressed in S-phase-arrested cells, and its levels do not change dramatically during the cell cycle, suggesting that proteolytic degradation rather than transcriptional controls ensure proper accumulation of Cdt1. Cdt1 can associate with the DNA replication inhibitor geminin, which is present in the S and G_2 phases of the cell cycle. Inhibition of DNA replication by geminin in cell-free DNA replication extracts can be reversed by the addition of excess Cdt1. Geminin may be responsible for preventing inappropriate origin firing by targeting Cdt1.

REFERENCES

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2. Wohlschlegel, J.A., et al. 2000. Inhibition of eukaryotic DNA replication by geminin binding to Cdt1. Science 290: 2309-2312.
3. Maiorano, D., et al. 2000. XCDT1 is required for the assembly of pre-replicative complexes in *Xenopus laevis*. Nature 404: 622-625.
4. Nishitani, H., et al. 2000. The Cdt1 protein is required to license DNA for replication in fission yeast. Nature 404: 625-628.
5. Nishitani, H., et al. 2001. The human licensing factor for DNA replication Cdt1 accumulates in G_1 and is destabilized after initiation of S-phase. J. Biol. Chem. 276: 44905-44911.
6. Gopalakrishnan, V., et al. 2001. Redundant control of rereplication in fission yeast. Proc. Natl. Acad. Sci. USA 98: 13114-13119.
7. Arentson, E., et al. 2002. Oncogenic potential of the DNA replication licensing protein CDT1. Oncogene 21: 1150-1158.
8. Spella, M., et al. 2007. Licensing regulators Geminin and Cdt1 identify progenitor cells of the mouse CNS in a specific phase of the cell cycle. Neuroscience 147: 373-387.

CHROMOSOMAL LOCATION

Genetic locus: Cdt1 (mouse) mapping to 8 E1.

PRODUCT

Cdt1 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 Cdt1 shRNA Plasmid (m): sc-142240-SH and Cdt1 shRNA (m) Lentiviral Particles: sc-142240-V as alternate gene silencing products.

For independent verification of Cdt1 (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-142240A, sc-142240B and sc-142240C.

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

Cdt1 siRNA (m) is recommended for the inhibition of Cdt1 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

Cdt1 (F-6): sc-365305 is recommended as a control antibody for monitoring of Cdt1 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 Cdt1 gene expression knockdown using RT-PCR Primer: Cdt1 (m)-PR: sc-142240-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.