SANTA CRUZ BIOTECHNOLOGY, INC.

Dbf4 siRNA (m): sc-37606

BACKGROUND

The Dbf4/Cdc7 protein kinase is essential for the activation of replication origins during S phase. Dbf4/Cdc7 efficiently phosphorylates several proteins that are required for the initiation of DNA replication, including five of the six minichromosome maintenance (MCM) proteins and the p180 subunit of DNA polymerase α-primase. This protein complex consists of the catalytic subunit Cdc7 associating with the regulatory and activating subunit Dbf4, and the kinase activity of the complex is regulated throughout the cell cycle mainly by fluctuating levels of Dbf4. Cdc7 is consistently expressed throughout the cell cycle, while the expression of Dbf4 is absent during G_1 phase and accumulates during S and G_2 phases. The anaphase-promoting complex rapidly degrades Dbf4 at the time of chromosome segregation, and the stability of Dbf4 remains low during pre-start G_1 phase. The coordinated degradation of Dbf4 and the time of chromosomes separation is important to ensuring that prereplicative complexes, which assemble after chromosome segregation, do not immediately refire.

REFERENCES

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3. Masai, H., et al. 1999. Cdc7 kinase complex as a molecular switch for DNA replication. Front. Biosci. 4: 834-840
4. Weinreich, M., et al. 1999. Dbf4p/Cdc7p kinase binds to chromatin during S phase and is regulated by both the APC and the RAD53 checkpoint pathway. EMBO J. 18: 5334-5346.
5. Jiang, W., et al. 1999. Mammalian Cdc7-Dbf4 protein kinase complex is essential for initiation of DNA replication. EMBO J. 18: 5703-5713.
6. Pasero, P., et al. 1999. A role for the Cdc7 kinase regulatory subunit Dbf4p in the formation of initiation-competent origins of replication. Genes Dev. 13: 2159-2176.
7. Ferreira, M.F., et al. 2000. Dbf4p, an essential S phase-promoting factor, is targeted for degradation by the anaphase-promoting complex. Mol. Cell. Biol. 20: 242-248.
8. Tsuji, T., et al. 2006. Essential role of phosphorylation of MCM2 by Cdc7/Dbf4 in the initiation of DNA replication in mammalian cells. Mol. Biol. Cell 17: 4459-4472.
9. Heffernan, T.P., et al. 2007. Cdc7-Dbf4 and the human S checkpoint response to UVC. J. Biol. Chem. 282: 9458-9468.

CHROMOSOMAL LOCATION

Genetic locus: Dbf4 (mouse) mapping to 5 A1.

PROTOCOLS

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

PRODUCT

Dbf4 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 Dbf4 shRNA Plasmid (m): sc-37606-SH and Dbf4 shRNA (m) Lentiviral Particles: sc-37606-V as alternate gene silencing products.

For independent verification of Dbf4 (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-37606A, sc-37606B and sc-37606C.

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

Dbf4 siRNA (m) is recommended for the inhibition of Dbf4 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Dbf4 gene expression knockdown using RT-PCR Primer: Dbf4 (m)-PR: sc-37606-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.