SANTA CRUZ BIOTECHNOLOGY, INC.

Dbf4b siRNA (h): sc-93916

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 alpha-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 also forms a complex with Dbf4b, a 615 amino acid nuclear protein. The Dbf4b/Cdc7 complex is required for progression of S and M phases of the cell cycle. Dbf4b is widely expressed, with highest expression found in testis. The expression of Dbf4b increases as cells enter S phase, and continues at a high rate through M phase. Dbf4b contains one BRCT domain and one DBF4-type zinc finger.

REFERENCES

1. Bousset, K., et al. 1998. The Cdc7 protein kinase is required for origin firing during S phase. Genes Dev. 12: 480-490
2. Lepke, M., et al. 1999. Identification, characterization and chromosomal localization of the cognate human and murine DBF4 genes. Mol. Gen. Genet. 262: 220-229.
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. Cdc7p-Dbf4p 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. Montagnoli, A., et al. 2002. Drf1, a novel regulatory subunit for human Cdc7 kinase. EMBO J. 21: 3171-3181.

CHROMOSOMAL LOCATION

Genetic locus: DBF4B (human) mapping to 17q21.31.

PRODUCT

Dbf4b siRNA (h) is a pool of 2 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 Dbf4b shRNA Plasmid (h): sc-93916-SH and Dbf4b shRNA (h) Lentiviral Particles: sc-93916-V as alternate gene silencing products.

For independent verification of Dbf4b (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-93916A and sc-93916B.

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

Dbf4b siRNA (h) is recommended for the inhibition of Dbf4b expression in human 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

Dbf4b (1A7): sc-517110 is recommended as a control antibody for monitoring of Dbf4b 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 Dbf4b gene expression knockdown using RT-PCR Primer: Dbf4b (h)-PR: sc-93916-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.