SANTA CRUZ BIOTECHNOLOGY, INC.

Cdc40 siRNA (h): sc-72837

BACKGROUND

Cell cycle events are regulated by the sequential activation and deactivation of cyclin dependent kinases (Cdks) and by the proteolysis of cyclins. The cell division control (Cdc) genes are required at various points in the cell cycle. Cdc40, also known as pre-mRNA-processing factor 17 (PRPF17) or EH-binding protein 3 (EHB3), is a 579 amino acid nuclear protein. Cdc40 is essential for the catalytic step II of the pre-mRNA splicing process, in which Cdc40 associates with the spliceosome C complex. Cdc40 contains seven WD repeats, which are important in protein-protein interactions. Cdc40 has sequence similarity to the yeast protein Prp17, which is involved in pre-mRNA splicing and cell cycle progression. The sequence similarity between the mammalian Cdc40 and the yeast Prp17 may indicate an additional role in cell cycle progression for mammalian Cdc40.

REFERENCES

1. Zhou, Z., et al. 1998. Human homologs of yeast prp16 and prp17 reveal conservation of the mechanism for catalytic step II of pre-mRNA splicing. EMBO J. 17: 2095-2106.
2. Lindsey, L. A., et al. 1998. Functional conservation of the human homolog of the yeast pre-mRNA splicing factor Prp17p. J. Biol. Chem. 273: 32771-32775.
3. Ben Yehuda, S., et al. 1998. Identification and functional analysis of hPRP17, the human homologue of the PRP17/CDC40 yeast gene involved in splicing and cell cycle control. RNA 4: 1304-1312.
4. Ben-Yehuda, S., et al. 2000. Genetic and physical interactions between factors involved in both cell cycle progression and pre-mRNA splicing in *Saccharomyces cerevisiae*. Genetics 156: 1503-1517.
5. Zhou, Z., et al. 2002. Comprehensive proteomic analysis of the human spliceosome. Nature 419: 182-185.
6. Jurica, M.S., et al. 2002. Purification and characterization of native spliceosomes suitable for three-dimensional structural analysis. RNA 8: 426-439.
7. Sapra, A.K., et al. 2004. Genome-wide analysis of pre-mRNA splicing: intron features govern the requirement for the second-step factor, Prp17 in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. J. Biol. Chem. 279: 52437-52446.

CHROMOSOMAL LOCATION

Genetic locus: CDC40 (human) mapping to 6q21.

PRODUCT

Cdc40 siRNA (h) 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 Cdc40 shRNA Plasmid (h): sc-72837-SH and Cdc40 shRNA (h) Lentiviral Particles: sc-72837-V as alternate gene silencing products.

For independent verification of Cdc40 (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-72837A, sc-72837B and sc-72837C.

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

Cdc40 siRNA (h) is recommended for the inhibition of Cdc40 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

Cdc40 (H-12): sc-398281 is recommended as a control antibody for monitoring of Cdc40 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 Cdc40 gene expression knockdown using RT-PCR Primer: Cdc40 (h)-PR: sc-72837-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.