

Cdc25B siRNA (h): sc-37552

BACKGROUND

The Cdc2/cyclin B enzyme, involved in regulation of mitosis in eukaryotic cells, is subject to multiple levels of control. Among these, the regulation of the catalytic subunit by Tyrosine phosphorylation is the best understood. Tyrosine phosphorylation inhibits the Cdc2/cyclin B complex, while Tyrosine dephosphorylation, which occurs at the onset of mitosis, directly activates the pre-MPH complex. The Cdc25 gene serves as a rate-limiting mitotic activator, apparently due to its action as the Cdc2 Tyrosine phosphatase. In the absence of Cdc25, Cdc2 accumulates in a Tyrosine phosphorylated state. In addition, Cdc25 proteins from a variety of species have been shown to share a low degree of sequence similarity with other Tyrosine phosphatases. The Cdc25 gene family consists of at least three members that share approximately 40% identity in their most conserved carboxy-terminal sequences.

REFERENCES

1. Murray, A.W., et al. 1989. Dominoes and clocks: the union of two views of the cell cycle. *Science* 246: 614-621.
2. Gould, K. and Nurse, P. 1989. Tyrosine phosphorylation of the fission Cdc2 protein kinase regulates entry into mitosis. *Nature* 342: 39-45.
3. Doree, M. 1990. Control of M phase by maturation promoting factor. *Curr. Opin. Cell Biol.* 2: 269-273.
4. Jessus, C., et al. 1990. Direct activation of Cdc2 with phosphatase: identification of p13^{Suc1}-sensitive and insensitive steps. *FEBS Lett.* 266: 4-8.
5. Boutros, R., et al. 2007. Cdc25B involvement in the centrosome duplication cycle and in microtubule nucleation. *Cancer Res.* 67: 11557-11564.

CHROMOSOMAL LOCATION

Genetic locus: CDC25B (human) mapping to 20p13.

PRODUCT

Cdc25B 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cdc25B shRNA Plasmid (h): sc-37552-SH and Cdc25B shRNA (h) Lentiviral Particles: sc-37552-V as alternate gene silencing products.

For independent verification of Cdc25B (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-37552A, sc-37552B and sc-37552C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Cdc25B siRNA (h) is recommended for the inhibition of Cdc25B 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 μ M in 66 μ l. 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

Cdc25B (DCS-162): sc-56266 is recommended as a control antibody for monitoring of Cdc25B 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Cdc25B gene expression knockdown using RT-PCR Primer: Cdc25B (h)-PR: sc-37552-PR (20 μ l, 593 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liu, X., et al. 2017. The E3 ubiquitin ligase APC/CCdh1 degrades MCPH1 after MCPH1- β TrCP2-Cdc25A-mediated mitotic entry to ensure neurogenesis. *EMBO J.* 36: 3666-3681.

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.