



Cdc25A siRNA (m): sc-35037

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

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5. Moreno, S., et al. 1990. Regulation of mitosis by cyclic accumulation of p80^{Cdc25} mitotic inducer in fission yeast. *Nature* 344: 549-552.
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8. Gautier, J., et al. 1991. Cdc25 is a specific tyrosine phosphatase that directly activates p34^{Cdc2}. *Cell* 67: 197-211.
9. Galaktionov, K., et al. 1991. Specific activation of cdc25 tyrosine phosphatases by B-type cyclins: evidence for multiple roles of mitotic cyclins. *Cell* 67: 1181-1194.

CHROMOSOMAL LOCATION

Genetic locus: Cdc25a (mouse) mapping to 9 F2.

PRODUCT

Cdc25A siRNA (m) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cdc25A shRNA Plasmid (m): sc-35037-SH and Cdc25A shRNA (m) Lentiviral Particles: sc-35037-V as alternate gene silencing products.

For independent verification of Cdc25A (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-35037A and sc-35037B.

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

Cdc25A siRNA (m) is recommended for the inhibition of Cdc25A 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 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

Cdc25A (F-6): sc-7389 is recommended as a control antibody for monitoring of Cdc25A 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 Cdc25A gene expression knockdown using RT-PCR Primer: Cdc25A (m)-PR: sc-35037-PR (20 μ l, 587 bp). 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.