



Cdc45 siRNA (m): sc-35045

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 cycle (Cdc) genes are required at various points in the cell cycle. Cdc25A, Cdc25B and Cdc25C protein tyrosine phosphatases function as mitotic activators by dephosphorylating Cdc2 p34 on regulatory tyrosine residues. Cdc6 and Cdc45 are the mammalian homologs of *S. cerevisiae* Cdc6 and Cdc45, which are involved in the initiation of DNA replication. Cdc37 appears to facilitate Cdk4/cyclin D1 complex formation and has been shown to form a stable complex with HSP 90. Cdc34, Cdc27 and Cdc16 function as ubiquitin-conjugating enzymes. Cdc34 is thought to be the structural and functional homolog of *S. cerevisiae* Cdc34, which is essential for the G₁ to S phase transition. Cdc16 and Cdc27 are components of the APC (anaphase-promoting complex) which ubiquitinates cyclin B, resulting in cyclin B/Cdk complex degradation.

REFERENCES

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- Gautier, J., et al. 1991. Cdc25 is a specific tyrosine phosphatase that directly activates p34^{Cdc2}. *Cell* 67: 197-211.
- Plon, S.E., et al. 1993. Cloning of the human homolog of the Cdc34 cell cycle gene by complementation in yeast. *Proc. Natl. Acad. Sci. USA* 90: 10484-10488.
- King, R.W., et al. 1995. A 20S complex containing Cdc27 and Cdc16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* 81: 279-288.
- Barinaga, M. 1995. A new twist to the cell cycle. *Science* 269: 631-632.
- Stepanova, L., et al. 1996. Mammalian p50^{Cdc37} is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. *Genes Dev.* 10: 1491-1502.
- Williams, R.S., et al. 1997. A human protein related to yeast Cdc6p. *Proc. Natl. Acad. Sci. USA* 94: 142-147.

CHROMOSOMAL LOCATION

Genetic locus: Cdc45l (mouse) mapping to 16 A3.

PRODUCT

Cdc45 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cdc45 shRNA Plasmid (m): sc-35045-SH and Cdc45 shRNA (m) Lentiviral Particles: sc-35045-V as alternate gene silencing products.

For independent verification of Cdc45 (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-35045A, sc-35045B and sc-35045C.

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

Cdc45 siRNA (m) is recommended for the inhibition of Cdc45 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

Cdc45 (G-12): sc-55569 is recommended as a control antibody for monitoring of Cdc45 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 Cdc45 gene expression knockdown using RT-PCR Primer: Cdc45 (m)-PR: sc-35045-PR (20 μ l, 583 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.