

Cdc34B siRNA (h): sc-105193

BACKGROUND

The eukaryotic cell division cycle consists of a number of gene-controlled sequences that involve cyclin dependent kinases (Cdks) and cell division control (Cdc) proteins. Cdc34B, also known as UBE2R2 (Ubiquitin-conjugating enzyme E2 R2) or UBC3B, is a 238 amino acid member of the E2 ubiquitin-conjugating enzyme family. Similar to Cdc34, Cdc34B functions to catalytically attach ubiquitin to various proteins, such as β -TrCP (an F-box protein that mediates β -catenin degradation), via an ATP-dependent reaction that yields AMP, a diphosphate and a ubiquitin-tagged protein. Cdc34B can be phosphorylated by the protein kinase CK2 (Casein kinase II), thereby allowing Cdc34B to regulate β -TrCP substrate recognition and, ultimately, enhance β -catenin degradation. Due to its ability to control β -TrCP activity, Cdc34B is thought to play a key role in cell cycle progression.

REFERENCES

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- Gautier, J., et al. 1991. Cdc25 is a specific tyrosine phosphatase that directly activates Cdc2 p34. *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 p50Cdc37 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: UBE2R2 (human) mapping to 9p13.3.

PRODUCT

Cdc34B 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 Cdc34B shRNA Plasmid (h): sc-105193-SH and Cdc34B shRNA (h) Lentiviral Particles: sc-105193-V as alternate gene silencing products.

For independent verification of Cdc34B (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-105193A, sc-105193B and sc-105193C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

Cdc34B siRNA (h) is recommended for the inhibition of Cdc34B 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

Cdc34B (E-6): sc-376097 is recommended as a control antibody for monitoring of Cdc34B 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 Cdc34B gene expression knockdown using RT-PCR Primer: Cdc34B (h)-PR: sc-105193-PR (20 μ l). 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.