SANTA CRUZ BIOTECHNOLOGY, INC.

Cdc4 siRNA (m): sc-37548

BACKGROUND

The F-box protein family is characterized by an approximately 40 amino acid motif known as the F-box. F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination. One family member, Cdc4, also known as AGO, FBW7, FBXW7, FBX30, SEL10, and FLJ11071, maps to human chromosome 4q31.3. Alternative splicing of this gene generates four transcript variants. In addition to an F-box, Cdc4 contains seven tandem WD40 repeats. Cdc4 binds directly to cyclin E and targets cyclin E for ubiquitin-mediated degradation. Mutations of the Cdc4 gene are detected in ovarian and breast cancer cell lines, suggesting that the gene may be involved in the pathogenesis of human cancers.

REFERENCES

1. Moberg, K., et al. 2001. Archipelago regulates cyclin E levels in *Drosophilia* and is mutated in human cancer cell lines. Nature 413: 268-269.
2. Strohmaier, H., et al. 2001. Human F-box protein hCdc4 targets cyclin E for proteolysis and is mutated in a breast cancer cell line. Nature 413: 316-322.
3. Koepp, D., et al. 2001. Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. Science 294: 173-177.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606278. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Tsunematsu, R., et al. 2004. Mouse Fbw7/Sel-10/Cdc4 is required for Notch degradation during vascular development. J. Biol. Chem. 279: 9417-9423.
6. LocusLink Report (LocusID: 55294). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: Fbxw7 (mouse) mapping to 3 F1.

PRODUCT

Cdc4 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cdc4 shRNA Plasmid (m): sc-37548-SH and Cdc4 shRNA (m) Lentiviral Particles: sc-37548-V as alternate gene silencing products.

For independent verification of Cdc4 (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-37548A, sc-37548B and sc-37548C.

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.

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

Cdc4 siRNA (m) is recommended for the inhibition of Cdc4 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 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

Cdc4 (3D1): sc-293423 is recommended as a control antibody for monitoring of Cdc4 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 Cdc4 gene expression knockdown using RT-PCR Primer: Cdc4 (m)-PR: sc-37548-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.