

CUL-4B siRNA (h): sc-37572

BACKGROUND

Cullin proteins comprise a distinct family of mediators that participate in the selective targeting of proteins for ubiquitin (Ub)-mediated proteolysis. Cul-1, which is the mammalian homolog of Cdc53 from yeast, is an integral component of the E3 ubiquitin ligase complex designated SCF. The SCF (Skp1/Cul-1/F-box protein complex) consists of Skp1 associating with both Cul-1 and an F-box protein, such as Skp2, which determines the substrate specificity of the complex. Cul-1 mediated ubiquitination results in the degradation of cell cycle proteins cyclin D, p21 and cyclin E. Another cullin, CUL-3 facilitates the degradation of cyclin E independent of SCF activity, while CUL-2 associates with the tumor suppressing protein VHL and elongin B to form VBC complexes, which structurally resemble the SCF ligase. Proteolysis also occurs by way of CUL-4A and CUL-4B associating with Nedd-8, a ubiquitin-like protein, where they too function as active components of a multifunctional E3 complex. CUL-5, or vasopressin-activated, calcium-mobilizing protein (VACM-1), is also included in the cullin family as it shares substantial sequence homology with CUL-1.

REFERENCES

1. Kipreos, E.T., et al. 1996. CUL-1 is required for cell cycle exit in *C. elegans* and identifies a novel gene family. *Cell* 85: 829-839.
2. Byrd, P.J., et al. 1997. Identification and analysis of expression of human VACM-1, a cullin gene family member located on chromosome 11q22-23. *Genome Res.* 7: 71-75.
3. Yu, Z.K., et al. 1998. Human CUL-1 associates with the Skp1/Skp2 complex and regulates p21^{CIP1/WAF1} and cyclin D proteins. *Proc. Natl. Acad. Sci. USA* 95: 11324-11329.
4. Chen, L.C., et al. 1998. The human homologue for the *Caenorhabditis elegans* CUL-4 gene is amplified and overexpressed in primary breast cancers. *Cancer Res.* 58: 3677-3683.
5. Tyers, M., et al. 1999. One ring to rule a superfamily of E3 ubiquitin ligases. *Science* 284: 601, 603-604.
6. Singer, J.D., et al. 1999. Cullin-3 targets cyclin E for ubiquitination and controls S phase in mammalian cells. *Genes Dev.* 13: 2375-2387.

CHROMOSOMAL LOCATION

Genetic locus: CUL4B (human) mapping to Xq24.

PRODUCT

CUL-4B 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 CUL-4B shRNA Plasmid (h): sc-37572-SH and CUL-4B shRNA (h) Lentiviral Particles: sc-37572-V as alternate gene silencing products.

For independent verification of CUL-4B (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-37572A, sc-37572B and sc-37572C.

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

CUL-4B siRNA (h) is recommended for the inhibition of CUL-4B 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

CUL-4 (H-11): sc-377188 is recommended as a control antibody for monitoring of CUL-4B 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 CUL-4B gene expression knockdown using RT-PCR Primer: CUL-4B (h)-PR: sc-37572-PR (20 μ l, 425 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.