SANTA CRUZ BIOTECHNOLOGY, INC.

CUL-5 siRNA (m): sc-37575

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-4 associating with NEDD8, a ubiquitin-like protein, where it too functions as an active component 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: Cul5 (mouse) mapping to 9 A5.3.

PRODUCT

CUL-5 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 CUL-5 shRNA Plasmid (m): sc-37575-SH and CUL-5 shRNA (m) Lentiviral Particles: sc-37575-V as alternate gene silencing products.

For independent verification of CUL-5 (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-37575A, sc-37575B and sc-37575C.

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

CUL-5 siRNA (m) is recommended for the inhibition of CUL-5 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

CUL-5 (F-6): sc-373822 is recommended as a control antibody for monitoring of CUL-5 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 CUL-5 gene expression knockdown using RT-PCR Primer: CUL-5 (m)-PR: sc-37575-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chadha, A., et al. 2015. Suppressive role of neddylation in dendritic cells during *Mycobacterium tuberculosis* infection. Tuberculosis 95: 599-607.

RESEARCH USE

For research use only, not for use in diagnostic procedures.