

CUL-4A siRNA (m): sc-60470

BACKGROUND

CUL-4A is a member of the cullin family of proteins that is involved in the ubiquitin-mediated degradation of cell cycle regulators. CUL-4A regulates cell cycle progression during differentiation, and overexpression of this protein significantly increases the number of cells in S phase and reduces the number that accumulate in G₀/G₁ phase. CUL-4A localizes to the cytoplasm where it stimulates ubiquitylation and degradation of the HoxA9 homeodomain protein, a key regulator of hematopoiesis and embryonic development. CUL-4A also stimulates the degradation of the damaged DNA-binding protein (DDB) that plays a role in DNA repair and is involved in the repair deficiency disease xeroderma pigmentosum. The CUL-4A gene is amplified and overexpressed in breast cancer, implicating the protein in tumorigenesis and/or tumor progression.

REFERENCES

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2. Osaka, F., et al. 1998. A new NEDD8-ligating system for cullin-4A. *Genes Dev.* 12: 2263-2268.
3. Shiyonov, P., et al. 2000. Cullin-4A associates with the UV-damaged DNA-binding protein DDB. *J. Biol. Chem.* 274: 35309-35312.
4. Chen, X., et al. 2001. UV-damaged DNA-binding proteins are targets of CUL-4A-mediated ubiquitination and degradation. *J. Biol. Chem.* 276: 48175-48182.
5. Gupta, A., et al. 2002. Study of the G₂/M cell cycle checkpoint overexpressing CUL-4A gene. *Int. J. Radiat. Oncol. Biol. Phys.* 52: 822-830.
6. Li, B., et al. 2002. CUL-4A is critical for early embryonic development. *Mol. Cell. Biol.* 22: 4997-5005.
7. Li, B., et al. 2003. Enforced expression of CUL-4A interferes with granulocytic differentiation and exit from the cell cycle. *Blood* 101: 1769-1776.
8. Zhang, Y., et al. 2003. CUL-4A stimulates ubiquitylation and degradation of the HoxA9 homeodomain protein. *EMBO J.* 22: 6057-6067.

CHROMOSOMAL LOCATION

Genetic locus: Cul4a (mouse) mapping to 8 A1.1.

PRODUCT

CUL-4A 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 CUL-4A shRNA Plasmid (m): sc-60470-SH and CUL-4A shRNA (m) Lentiviral Particles: sc-60470-V as alternate gene silencing products.

For independent verification of CUL-4A (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-60470A, sc-60470B and sc-60470C.

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-4A siRNA (m) is recommended for the inhibition of CUL-4A 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

CUL-4 (H-11): sc-377188 is recommended as a control antibody for monitoring of CUL-4A 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-4A gene expression knockdown using RT-PCR Primer: CUL-4A (m)-PR: sc-60470-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.

PROTOCOLS

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