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CUL-4A shRNA (h) Lentiviral Particles: sc-44355-V

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

- 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.
- Osaka, F., et al. 1998. A new NEDD8-ligating system for cullin-4A. *Genes Dev.* 12: 2263-2268.
- Shiyonov, P., et al. 2000. Cullin-4A associates with the UV-damaged DNA-binding protein DDB. *J. Biol. Chem.* 274: 35309-35312.
- 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.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: CUL4A (human) mapping to 13q34.

PRODUCT

CUL-4A shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CUL-4A siRNA (h): sc-44355 and CUL-4A shRNA Plasmid (h): sc-44355-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

CUL-4A shRNA (h) Lentiviral Particles is recommended for the inhibition of CUL-4A expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

CUL-4A (K-16): sc-50919 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CUL-4A gene expression knockdown using RT-PCR Primer: CUL-4A (h)-PR: sc-44355-PR (20 μ l, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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