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SANTA CRUZ BIOTECHNOLOGY, INC.

Ubp-M siRNA (h): sc-41687

BACKGROUND

Ubiquitin-processing protease-M (Ubp-M) belongs to a family of enzymes that regulate the degradation of ubiquitinated proteins by deubiquitination. Ubiquitin-mediated proteolysis requires the transfer of ubiquitin chains to lysine groups on selected cellular proteins, which then potentiates the proteolytic degradation of these conjugated substrates by the 26S proteasome. Ubps, which are also designated deubiquitinating enzymes (DUBs), regulate growth activity and differentiation. Ubp-M is localized to the cytosol and is phosphorylated during the G_2/M phase transition through the completion of mitosis. This phosphorylation state coincides with an accumulation of free ubiquitin chains within the cell and an increased hydrolysis of ubiquitin conjugated proteins. Targets of Ubp-M include the histone proteins H2A and H2B, which are monoubiquitinated during interphase and anaphase and are deubuiquitinated during mitoisis. This deubiquitination of the histone proteins correlates to the condensation of the mitotic chromatin, indicating that Ubp-M influences histone function and, thereby, facilitates the organization of mitotic chromatin and directs the progression of cell growth.

REFERENCES

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2. Hecht, A., et al. 1995. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell 80: 583-592.
3. Hochstrasser, M. 1995. Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr. Opin. Cell Biol. 7: 215-223.
4. Wilkinson, K.D., et al. 1995. Metabolism of the polyubiquitin degradation signal: structure, mechanism, and role of isopeptidase T. Biochemistry 34: 14535-14546.
5. Haas, A.L., et al. 1997. Pathways of ubiquitin conjugation. FASEB J. 11: 1257-1268.
6. Cai, S.Y., et al. 1999. A mutant deubiquitinating enzyme (Ubp-M) associates with mitotic chromosomes and blocks cell division. Proc. Natl. Acad. Sci. USA 96: 2828-2833.

CHROMOSOMAL LOCATION

Genetic locus: USP16 (human) mapping to 21q21.3.

PRODUCT

Ubp-M 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Ubp-M shRNA Plasmid (h): sc-41687-SH and Ubp-M shRNA (h) Lentiviral Particles: sc-41687-V as alternate gene silencing products.

For independent verification of Ubp-M (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-41687A, sc-41687B and sc-41687C.

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

Ubp-M siRNA (h) is recommended for the inhibition of Ubp-M 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 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

Ubp-M (B-3): sc-390683 is recommended as a control antibody for monitoring of Ubp-M 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 Ubp-M gene expression knockdown using RT-PCR Primer: Ubp-M (h)-PR: sc-41687-PR (20 µl). 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.