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SANTA CRUZ BIOTECHNOLOGY, INC.

UFD1 siRNA (h): sc-41689

BACKGROUND

Ubiquitin-mediated proteolysis requires the transfer of ubiquitin (Ub) to lysine groups on selected cellular proteins, which then potentiates the proteolytic degradation of these protein conjugates by the 26S Proteasome. Ub-fusions are cleaved by Ub-specific processing proteases (UBps) or alternatively by the Ub-fusion degradation (UFD) pathway. The UBP pathway targets the C-terminal glycine residue on Ub that is involved in the formation of Ub-conjugates, while UFD proteins preferentially cleave Ub-conjugated proteins that contain an amino acid substitution at this glycine residue. The UFD1 protein was originally characterized in the yeast *S. cerevisiae* and subsequently, the human homolog UFD1 or UFD1L was identified. *In vitro,* UFD1 attenuates the degradation of Ub-fusions, which have a proline or valine residue substituted at the Gly76 moiety, by the selective multiubiquitination of the Ub chain of the Ub-conjugate. Mutations within the UFD1 gene are implicated in the development of CATCH22 syndrome, which is characterized by cardiac defects, cleft palate and hypocalcemia, suggesting that this proteolytic pathway may be involved in the progression of these developmental defects.

REFERENCES

1. Jentsch, S. 1992. The ubiquitin-conjugation system. Annu. Rev. Genet. 26: 179-207.
2. Johnson, E.S., et al. 1995. A proteolytic pathway that recognizes ubiquitin as a degradation signal. J. Biol. Chem. 270: 17442-17456.
3. Hochstrasser, M. 1995. Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. Curr. Opin. Cell Biol. 7: 215-223.
4. Haas, A.L., et al. 1997. Pathways of ubiquitin conjugation. FASEB J. 11: 1257-1268.

CHROMOSOMAL LOCATION

Genetic locus: UFD1L (human) mapping to 22q11.21.

PRODUCT

UFD1 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 UFD1 shRNA Plasmid (h): sc-41689-SH and UFD1 shRNA (h) Lentiviral Particles: sc-41689-V as alternate gene silencing products.

For independent verification of UFD1 (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-41689A, sc-41689B and sc-41689C.

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

UFD1 siRNA (h) is recommended for the inhibition of UFD1 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

UFD1 (B-7): sc-377265 is recommended as a control antibody for monitoring of UFD1 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 UFD1 gene expression knockdown using RT-PCR Primer: UFD1 (h)-PR: sc-41689-PR (20 µl, 597 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lin, P.H., et al. 2008. Ubiquitin-proteasome system mediates heme oxygenase-1 degradation through endoplasmic reticulum-associated degradation pathway. Biochim. Biophys. Acta 1783: 1826-1834.
2. Chen, S.F., et al. 2013. Caveolin-1 interacts with Derlin-1 and promotes ubiquitination and degradation of cyclooxygenase-2 via collaboration with p97 complex. J. Biol. Chem. 288: 33462-33469.
3. Nguyen, T.V., et al. 2017. p97/VCP promotes degradation of CRBN substrate glutamine synthetase and neosubstrates. Proc. Natl. Acad. Sci. USA 114: 3565-3571.

RESEARCH USE

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