

UCH-L5 siRNA (h): sc-76797

BACKGROUND

As a component of the 19S regulatory complex of the 26S Proteasome, UCH-L5 (ubiquitin carboxyl-terminal hydrolase isozyme L5), also known as UCH37, is a 329 amino acid protein that functions to edit polyubiquitinated protein substrates. Since UCH-L5 has the potential to rescue ubiquitinated proteins, including oncogenic proteins, from proteasomal degradation, it is likely that deregulation of UCH-L5 may affect tumor growth. Through associations with Smad7, UCH-L5 can dramatically upregulate TGF β -dependent gene expression by de-ubiquitinating and stabilizing TGF β RI. Also, since overexpression of UCH-L5 and other deubiquitinating enzymes has been observed in many cancer cell lines, inhibition of these proteins may be of some interest in designing therapies for cancer treatment. There are four isoforms of UCH-L5 that exist as a result of alternative splicing events.

REFERENCES

1. Lai, C.H., et al. 2000. Identification of novel human genes evolutionarily conserved in *Caenorhabditis elegans* by comparative proteomics. *Genome Res.* 10: 703-713.
2. Wicks, S.J., et al. 2006. Reversible ubiquitination regulates the Smad/TGF- β signalling pathway. *Biochem. Soc. Trans.* 34: 761-763.
3. Hamazaki, J., et al. 2006. A novel proteasome interacting protein recruits the deubiquitinating enzyme UCH37 to 26S proteasomes. *EMBO J.* 25: 4524-4536.

CHROMOSOMAL LOCATION

Genetic locus: UCHL5 (human) mapping to 1q31.2.

PRODUCT

UCH-L5 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UCH-L5 shRNA Plasmid (h): sc-76797-SH and UCH-L5 shRNA (h) Lentiviral Particles: sc-76797-V as alternate gene silencing products.

For independent verification of UCH-L5 (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-76797A, sc-76797B and sc-76797C.

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

UCH-L5 siRNA (h) is recommended for the inhibition of UCH-L5 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 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

UCH-L5 (C-4): sc-271002 is recommended as a control antibody for monitoring of UCH-L5 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 UCH-L5 gene expression knockdown using RT-PCR Primer: UCH-L5 (h)-PR: sc-76797-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liu, N., et al. 2014. Clinically used antirheumatic agent auranofin is a proteasomal deubiquitinase inhibitor and inhibits tumor growth. *Oncotarget* 5: 5453-5471.
2. Liu, N., et al. 2014. A novel proteasome inhibitor suppresses tumor growth via targeting both 19S proteasome deubiquitinases and 20S proteolytic peptidases. *Sci. Rep.* 4: 5240.
3. Liao, Y., et al. 2018. Growth arrest and apoptosis induction in androgen receptor-positive human breast cancer cells by inhibition of USP14-mediated androgen receptor deubiquitination. *Oncogene* 37: 1896-1910.
4. Liao, Y., et al. 2019. USP10 modulates the SKP2/Bcr-Abl axis via stabilizing SKP2 in chronic myeloid leukemia. *Cell Discov.* 5: 24.
5. Ramachandran, A., et al. 2021. Deubiquitination and activation of the NLRP3 inflammasome by UCHL5 in HCV-infected cells. *Microbiol. Spectr.* E-published.

RESEARCH USE

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