SANTA CRUZ BIOTECHNOLOGY, INC.

UCH-L5 siRNA (m): sc-76798

BACKGROUND

As a component of the 19S regulatory complex of the 26S proteosome, UCH-L5 (ubiquitin carboxyl-terminal hydrolase isozyme L5), also known as UCH37, is a 329 amino acid protein that functions to edit polyubiquinated protein substrates. Since UCH-L5 has the potential to rescue ubiquinated proteins, including oncogenic proteins, from proteasomal degradation, it is likely that deregulation of UCH-L5 may affect tumor growth. Through associations with Smad7, UHC-L5 can dramatically upregulate TGFβ-dependent gene expression by deubiquinating and stabilizing TGFβ 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.
4. Qiu, X.B., et al. 2006. hRpn13/ADRM1/GP110 is a novel proteasome subunit that binds the deubiquitinating enzyme, UCH37. EMBO J. 25: 5742-5753.
5. Yao, T., et al. 2006. Proteasome recruitment and activation of the Uch37 deubiquitinating enzyme by Adrm1. Nat. Cell Biol. 8: 994-1002.
6. Horton, R.A., et al. 2007. A substrate for deubiquitinating enzymes based on time-resolved fluorescence resonance energy transfer between terbium and yellow fluorescent protein. Anal. Biochem. 360: 138-143.
7. Liu, C.H., et al. 2007. New insights into the role of the ubiquitin-proteasome pathway in the regulation of apoptosis. Chang Gung Med. J. 30: 469-479.

CHROMOSOMAL LOCATION

Genetic locus: Uchl5 (mouse) mapping to 1 F.

PRODUCT

UCH-L5 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UCH-L5 shRNA Plasmid (m): sc-76798-SH and UCH-L5 shRNA (m) Lentiviral Particles: sc-76798-V as alternate gene silencing products.

For independent verification of UCH-L5 (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-76798A, sc-76798B and sc-76798C.

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

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

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-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 UCH-L5 gene expression knockdown using RT-PCR Primer: UCH-L5 (m)-PR: sc-76798-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.