SANTA CRUZ BIOTECHNOLOGY, INC.

UBE2L3 siRNA (h): sc-61746

BACKGROUND

The ubiquitin (Ub) pathway involves three sequential enzymatic steps that facilitate the conjugation of Ub and Ub-like molecules to specific protein substrates. The first step requires the ATP-dependent activation of the Ub C-terminus and the assembly of multi-Ub chains by the Ub-activating enzyme known as the E1 component. The Ub chain is then conjugated to the Ub-conjugating enzyme (E2) to generate an intermediate Ub-E2 complex. The Ub-ligase (E3) then catalyzes the transfer of Ub from E2 to the appropriate protein substrate. UBE2E1 and UBE2L3, also designated UBCH6 and UBCH7 respectively in human, are E2 conjugating enzymes that interact with various proteins. Specifically, UBE2E1 interacts with the tumor suppressor protein TSSC5. UBE2L3 has been shown to mediate c-Fos degradation, NFκB maturation, human papilloma virus-mediated p53 and Myc protein degradation.

REFERENCES

1. Nuber, U., et al. 1996. Cloning of human ubiquitin-conjugating enzymes UBCH6 and UBCH7 (E2-F1) and characterization of their interaction with E6-AP and RSP5. J. Biol. Chem. 271: 2795-800.
2. Ardley, H.C., et al. 2000. Promoter analysis of the human ubiquitin-conjugating enzyme including UBE2L3 which encodes UBCH7. Biochim. Biophys. Acta 1491: 57-64.
3. Ardley, H.C., et al. 2001. Features of the Parkin/ariadne-like ubiquitin ligase, its interaction with the ubiquitin-conjugating enzyme, UBCH7. J. Biol. Chem. 276: 19640-19647.
4. Passmore, L.A., et al. 2004. Getting into position: the catalytic mechanisms of protein ubiquitylation. Biochem. J. 379: 513-525.
5. Kuhlbrodt, K., et al. 2005. Orchestra for assembly and fate of polyubiquitin chains. Essays Biochem. 41: 1-14.
6. Takeuchi, T., et al. 2006. Link between the ubiquitin conjugation system and the ISG15 conjugation system: ISG15 conjugation to the UBCH6 ubiquitin E2 enzyme. J. Biochem. 138: 711-719.
7. Yamada, H.Y. and Gorbsky, G.J. 2006. Tumor suppressor candidate TSSC5 is regulated by UbcH6 and a novel ubiquitin ligase RING105. Oncogene 25: 1330-1339.

CHROMOSOMAL LOCATION

Genetic locus: UBE2L3 (human) mapping to 22q11.21.

PRODUCT

UBE2L3 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 UBE2L3 shRNA Plasmid (h): sc-61746-SH and UBE2L3 shRNA (h) Lentiviral Particles: sc-61746-V as alternate gene silencing products.

For independent verification of UBE2L3 (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-61746A, sc-61746B and sc-61746C.

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

UBE2L3 siRNA (h) is recommended for the inhibition of UBE2L3 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

UBE2L3 (B-11): sc-390032 is recommended as a control antibody for monitoring of UBE2L3 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 UBE2L3 gene expression knockdown using RT-PCR Primer: UBE2L3 (h)-PR: sc-61746-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.