SANTA CRUZ BIOTECHNOLOGY, INC.

UBE2G1 siRNA (h): sc-93745

BACKGROUND

Ubiquitin is an abundant, highly conserved protein found in all eukaryotic cells either free or covalently attached to cellular proteins. The primary function of ubiquitin in mammalian systems is to clear abnormal, foreign, and improperly folded proteins by targeting them for proteosome degradation. In *Saccharomyces cerevisiae,* ubiquitin-like proteins include Rub1, Ula1, Uba3, Smt3, Ubc2, Ubc12 and Ubc9. Rub1 shares 53% homology with ubiquitin and requires activation via the E2 proteins, including Ula1, Uba3 and Ubc12 in order to conjugate to substrates directed to different proteolytic systems. Ubc4 catalyzes ubiquitination of IκBα in a phosphorylation and SCFB-TRCP dependent manner. In this particular reaction, E1 first transfers ubiquitin to the E2 component Ubc4, and Ubc4 then associates with E3 ligase, which conjugates the poly-ubiquitin chain on a target protein. In this fashion, the chain tags the IκBα for degradation by a proteasome thus lifting the inhibitory effect of IκBα on NFκB and allowing NFκB to enter the nucleus.

REFERENCES

1. Ciechanover, A. 1994. The ubiquitin-proteasome proteolytic pathway. Cell 79: 13-21.
2. Ciechanover, A., et al. 1994. The ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins. FASEB J. 8: 182-191.
3. Hochstrasser, M. 1995. Ubiquitin, proteasomes and the regulation of intracellular protein degradation. Curr. Opin. Cell Biol. 7: 215-223.
4. Liakopoulos, D., et al. 1998. A novel protein modification pathway related to the ubiquitin system. EMBO J. 17: 2208-2214.
5. Schwarz, S.E., et al. 1998. The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme. Proc. Natl. Acad. Sci. USA 95: 560-564.
6. Gong, L., et al. 1999. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. J. Biol. Chem. 274: 12036-12042.
7. Raboy, B., et al. 1999. Heat-induced cell cycle arrest of *Saccharomyces cerevisiae:* involvement of the RAD6/UBC2 and WSC2 genes in its reversal. Mol. Microbiol. 32: 729-739.

CHROMOSOMAL LOCATION

Genetic locus: UBE2G1 (human) mapping to 17p13.2.

PRODUCT

UBE2G1 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 UBE2G1 shRNA Plasmid (h): sc-93745-SH and UBE2G1 shRNA (h) Lentiviral Particles: sc-93745-V as alternate gene silencing products.

For independent verification of UBE2G1 (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-93745A, sc-93745B and sc-93745C.

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

UBE2G1 siRNA (h) is recommended for the inhibition of UBE2G1 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

UBE2G1 (H-2): sc-398969 is recommended as a control antibody for monitoring of UBE2G1 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 UBE2G1 gene expression knockdown using RT-PCR Primer: UBE2G1 (h)-PR: sc-93745-PR (20 µl, 594 bp). 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.