

UBA3 siRNA (h): sc-76783

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 proteasome degradation. UBA3 (ubiquitin-like modifier activating enzyme 3), also known as NEDD8-activating enzyme E1 catalytic subunit or UBE1C (ubiquitin-activating enzyme E1C), is a 463 amino acid protein belonging to the ubiquitin-activating E1 family and UBA3 subfamily. Ubiquitously expressed, UBA3 acts as an activator to NEDD8, a ubiquitin-like protein, thus regulating cell division, signaling and embryogenesis. UBA3 exists as two isoforms due to alternative splicing events.

REFERENCES

1. Ciechanover, A. 1994. The ubiquitin-proteasome proteolytic pathway. *Cell* 79: 13-21.
2. Ciechanover, A. and Schwartz, A.L. 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. Bai, C., et al. 1996. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 86: 263-274.
5. Liakopoulos, D., et al. 1998. A novel protein modification pathway related to the ubiquitin system. *EMBO J.* 17: 2208-2214.
6. Gong, L. and Yeh, E.T. 1999. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. *J. Biol. Chem.* 274: 12036-12042.

CHROMOSOMAL LOCATION

Genetic locus: UBA3 (human) mapping to 3p14.1.

PRODUCT

UBA3 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 UBA3 shRNA Plasmid (h): sc-76783-SH and UBA3 shRNA (h) Lentiviral Particles: sc-76783-V as alternate gene silencing products.

For independent verification of UBA3 (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-76783A, sc-76783B and sc-76783C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

UBA3 siRNA (h) is recommended for the inhibition of UBA3 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

UBA3 (E-5): sc-377272 is recommended as a control antibody for monitoring of UBA3 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 UBA3 gene expression knockdown using RT-PCR Primer: UBA3 (h)-PR: sc-76783-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. Xu, D., et al. 2021. Cullin 2-RBX1 E3 ligase and USP2 regulate antithrombin ubiquitination and stability. *FASEB J.* 35: e21800.

RESEARCH USE

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