

UBE2J1 siRNA (m): sc-154853

BACKGROUND

Ubiquitination is an important molecular mechanism by which abnormal or short-lived proteins are targeted for degradation by the concerted efforts of at least three classes of enzymes: ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s) and ubiquitin-protein ligases (E3s). UBE2J1 (ubiquitin-conjugating enzyme E2 J1), also known as Ubc6p, CGI-76, NCUBE1, HSPC153 or HSPC205, is a 318 amino acid single-pass type IV membrane protein that belongs to the E2 ubiquitin-conjugating enzyme family and is involved in protein degradation. Localized to the membrane of the endoplasmic reticulum (ER), UBE2J1 catalyzes the attachment of ubiquitin to misfolded membrane proteins, thereby targeting them for proteasomal destruction. This ATP-dependent reaction yields AMP, a diphosphate and a ubiquitin-tagged protein and may be a method of quality control within the ER.

REFERENCES

1. Gilon, T., et al. 2000. Degradation signals recognized by the Ubc6p-Ubc7p ubiquitin-conjugating enzyme pair. *Mol. Cell. Biol.* 20: 7214-7219.
2. Lester, D., et al. 2000. Identification of a family of noncanonical ubiquitin-conjugating enzymes structurally related to yeast UBC6. *Biochem. Biophys. Res. Commun.* 269: 474-480.
3. Walter, J., et al. 2001. Sec61p-independent degradation of the tail-anchored ER membrane protein Ubc6p. *EMBO J.* 20: 3124-3131.
4. Tiwari, S. and Weissman, A.M. 2001. Endoplasmic reticulum (ER)-associated degradation of T cell receptor subunits. Involvement of ER-associated ubiquitin-conjugating enzymes (E2s). *J. Biol. Chem.* 276: 16193-16200.
5. Botero, D., et al. 2002. Ubc6p and ubc7p are required for normal and substrate-induced endoplasmic reticulum-associated degradation of the human selenoprotein type 2 iodothyronine monodeiodinase. *Mol. Endocrinol.* 16: 1999-2007.
6. Lenk, U., et al. 2002. A role for mammalian Ubc6 homologues in ER-associated protein degradation. *J. Cell Sci.* 115: 3007-3014.
7. Wu, C.J., et al. 2005. TNF- α induced c-IAP1/TRAF2 complex translocation to a Ubc6-containing compartment and TRAF2 ubiquitination. *EMBO J.* 24: 1886-1898.

CHROMOSOMAL LOCATION

Genetic locus: Ube2j1 (mouse) mapping to 4 A5.

PRODUCT

UBE2J1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBE2J1 shRNA Plasmid (m): sc-154853-SH and UBE2J1 shRNA (m) Lentiviral Particles: sc-154853-V as alternate gene silencing products.

For independent verification of UBE2J1 (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-154853A, sc-154853B and sc-154853C.

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

UBE2J1 siRNA (m) is recommended for the inhibition of UBE2J1 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 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

UBE2J1 (B-6): sc-377002 is recommended as a control antibody for monitoring of UBE2J1 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 UBE2J1 gene expression knockdown using RT-PCR Primer: UBE2J1 (m)-PR: sc-154853-PR (20 μ l). 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.