



## UBE2D4 siRNA (m): sc-41684

### 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 proteosomal degradation. UBE2D proteins, including UBE2D1 (ubiquitin-conjugating enzyme E2D1), UBE2D2 (ubiquitin-conjugating enzyme E2D2), UBE2D3 (ubiquitin-conjugating enzyme E2D3) and UBE2D4 (ubiquitin-conjugating enzyme E2D4, also known as HBUCE1), are E2 ubiquitin-conjugating enzymes that catalyze the ubiquitination of target proteins in an ATP-dependent manner. Specifically, an E1 enzyme first transfers a ubiquitin residue to an E2 component (a UBE2D protein), and the UBE2D protein then associates with an E3 ubiquitin-protein ligase, which immediately transfers that residue to a protein that is targeted for degradation.

### REFERENCES

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- 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.
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### CHROMOSOMAL LOCATION

Genetic locus: OTTMUSG00000020733 (mouse) mapping to X A7.3.

### PRODUCT

UBE2D4 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBE2D4 shRNA Plasmid (m): sc-41684-SH and UBE2D4 shRNA (m) Lentiviral Particles: sc-41684-V as alternate gene silencing products.

For independent verification of UBE2D4 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3 nmol of lyophilized siRNA. These include: sc-41684A, sc-41684B and sc-41684C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

UBE2D4 siRNA (m) is recommended for the inhibition of UBE2D4 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  $\mu$ M in 60  $\mu$ 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

UBE2D (A-15): sc-14998 is recommended as a control antibody for monitoring of UBE2D 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor UBE2D gene expression knockdown using RT-PCR Primer: UBE2D4 (m)-PR: sc-41684-PR (20  $\mu$ 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.