

UBE2C siRNA (m): sc-61743

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. UBE2C, also designated UBCH10 in human, is an E2 ubiquitin conjugating enzyme for the anaphase promoting complex (APC), which coordinates mitosis and G₁ by sequentially promoting the degradation of key cell-cycle regulators. UBE2C is overexpressed in many different types of cancers and may be a potential therapeutic target.

REFERENCES

1. Lin, Y., et al. 2002. Structural and functional analysis of the human mitotic-specific ubiquitin-conjugating enzyme, UBCH10. *J. Biol. Chem.* 277: 21913-21921.
2. Okamoto, Y., et al. 2003. UBCH10 is the cancer-related E2 ubiquitin-conjugating enzyme. *Cancer Res.* 63: 4167-4173.
3. Rape, M., et al. 2004. Autonomous regulation of the anaphase-promoting complex couples mitosis to S-phase entry. *Nature* 432: 588-595.
4. Passmore, L.A., et al. 2004. Getting into position: the catalytic mechanisms of protein ubiquitylation. *Biochem. J.* 379: 513-525.
5. Wagner, K.W., et al. 2004. Overexpression, genomic amplification and therapeutic potential of inhibiting the UBCH10 ubiquitin conjugase in human carcinomas of diverse anatomic origin. *Oncogene* 23: 6621-6629.
6. Kobirumaki, F., et al. 2005. A novel UBCH10-binding protein facilitates the ubiquitylation of cyclin B *in vitro*. *J. Biochem.* 137:133-139.
7. Kuhlbrodt, K., et al. 2005. Orchestra for assembly and fate of polyubiquitin chains. *Essays Biochem.* 41: 1-14.
8. Rape, M., et al. 2006. The processivity of multiubiquitination by the APC determines the order of substrate degradation. *Cell* 124: 89-103.

CHROMOSOMAL LOCATION

Genetic locus: Ube2c (mouse) mapping to 2 H3.

PRODUCT

UBE2C 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 UBE2C shRNA Plasmid (m): sc-61743-SH and UBE2C shRNA (m) Lentiviral Particles: sc-61743-V as alternate gene silencing products.

For independent verification of UBE2C (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-61743A, sc-61743B and sc-61743C.

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

UBE2C siRNA (m) is recommended for the inhibition of UBE2C 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

UBE2C (B-12): sc-166339 is recommended as a control antibody for monitoring of UBE2C 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 UBE2C gene expression knockdown using RT-PCR Primer: UBE2C (m)-PR: sc-61743-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.