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UBE2E2 siRNA (m): sc-154846

BACKGROUND

Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). The first step in the ubiquitination process requires the ATP-dependent activation of the ubiquitin C-terminus and the assembly of multi-ubiquitin chains by the E1 enzyme. The ubiquitin chain is then conjugated to the E2 enzyme to generate an intermediate ubiquitin-E2 complex. The E3 enzyme then catalyzes the transfer of ubiquitin from E2 to the appropriate protein substrate, thereby targeting that substrate for degradation. A wide range of enzymes facilitate this proteolytic ubiquitin pathway, one of which is UBE2E2 (also known as UBCH8 in human), which functions as an E2 enzyme and catalyzes the ATP-dependent covalent attachment of ubiquitin to target proteins, thereby playing an important role in protein degradation.

REFERENCES

- Kimura, M., et al. 1997. cDNA cloning, characterization, and chromosome mapping of UBE2E2 encoding a human ubiquitin-conjugating E2 enzyme. *Cytogenet. Cell Genet.* 78: 107-111.
- Moynihan, T.P., et al. 1999. The ubiquitin-conjugating enzymes UbcH7 and UbcH8 interact with RING finger/IBR motif-containing domains of HHARI and H7-AP1. *J. Biol. Chem.* 274: 30963-30968.
- Ito, K., et al. 2001. N-Terminally extended human ubiquitin-conjugating enzymes (E2s) mediate the ubiquitination of RING-finger proteins, ARA54 and RNF8. *Eur. J. Biochem.* 268: 2725-2732.

CHROMOSOMAL LOCATION

Genetic locus: Ube2e2 (mouse) mapping to 14 A2.

PRODUCT

UBE2E2 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 UBE2E2 shRNA Plasmid (m): sc-154846-SH and UBE2E2 shRNA (m) Lentiviral Particles: sc-154846-V as alternate gene silencing products.

For independent verification of UBE2E2 (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-154846A, sc-154846B and sc-154846C.

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

UBE2E2 siRNA (m) is recommended for the inhibition of UBE2E2 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor UBE2E2 gene expression knockdown using RT-PCR Primer: UBE2E2 (m)-PR: sc-154846-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.