SANTA CRUZ BIOTECHNOLOGY, INC.

UBC12 siRNA (m): sc-76787

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). UBC12, also known as UBE2M (ubiquitin-conjugating enzyme E2M), hUbc12 or UBC-RS2, is a 183 amino acid member of the E2 ubiquitin-conjugating enzyme family. UBC12 is linked with NEDD8 (neural precursor cell expressed, developmentally down-regulated 8), a ubiquitin-like protein. Via this interaction, UBC12 facilitates the attachment of NEDD8 to proteins targeted for degradation. Due to its ability to control the conjugation of NEDD8 to cellular proteins, UBC12 is thought to play a role in cell proliferation events.

REFERENCES

1. Ciechanover, A., et al. 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.
2. Hochstrasser, M. 1995. Ubiquitin, proteasomes and the regulation of intracellular protein degradation. Curr. Opin. Cell Biol. 7: 215-223.
3. Osaka, F., et al. 1998. A new NEDD8-ligating system for cullin-4A. Genes Dev. 12: 2263-2268.
4. Gong, L., et al. 1999. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. J. Biol. Chem. 274: 12036-12042.
5. Wada, H., et al. 2000. A dominant-negative UBC12 mutant sequesters NEDD8 and inhibits NEDD8 conjugation *in vivo*. J. Biol. Chem. 275: 17008-17015.
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CHROMOSOMAL LOCATION

Genetic locus: Ube2m (mouse) mapping to 7 A1.

PRODUCT

UBC12 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBC12 shRNA Plasmid (m): sc-76787-SH and UBC12 shRNA (m) Lentiviral Particles: sc-76787-V as alternate gene silencing products.

For independent verification of UBC12 (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-76787A, sc-76787B and sc-76787C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

UBC12 siRNA (m) is recommended for the inhibition of UBC12 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 µM in 60 µl. 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

UBC12 (D-4): sc-390064 is recommended as a control antibody for monitoring of UBC12 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor UBC12 gene expression knockdown using RT-PCR Primer: UBC12 (m)-PR: sc-76787-PR (20 µl). 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.