

Mms2 siRNA (h): sc-72124

BACKGROUND

Uev1A (also designated ubiquitin-conjugating enzyme E2 variant 1 (UEV1) and CROC1) and Mms2 (UEV2) proteins are similar in sequence and in predicted structure to the ubiquitin-conjugating enzymes or E2s, but lack a critical cysteine residue essential for the catalytic activity of E2 enzymes. Therefore, Uev1A does not have ubiquitin-conjugating activity *in vitro*. However, constitutive expression of exogenous Uev1A in colon carcinoma cells inhibits their capacity to differentiate upon confluence. Studies on recombinant Uev1A show that it localizes to the nucleus, excluding the nucleolar regions. Uev1A functions with TRAF6, a RING domain protein, to catalyze the synthesis of unique polyubiquitin chains linked through Lysine 63 of ubiquitin. UBC13 (ubiquitin-conjugating enzyme E2N (UBE2N)) may be involved in protein degradation mainly in the muscles and testis. In yeast, Mms2/UBC13 complex assembles novel polyubiquitin chains for signaling in DNA repair, and suggests that UEV proteins may act to increase diversity and selectivity in ubiquitin conjugation.

REFERENCES

1. Rothfoks, M.L. and Lin, S.L. 1997. CROC-1 encodes a protein which mediates transcriptional activation of the human FOS promoter. *Gene* 195: 141-149.
2. Sancho, E., Vila, M.R., Sanchez-Pulido, L., Lozano, J.J., Paciucci, R., Nadal, M., Fox, M., Harvey, C., Bercovich, B., Loukili, N., Ciechanover, A., Lin, S., Sans, F., Estivill, X., Valencia, A. and Thomson, T.M. 1998. Role of UEV-1, an inactive variant of the E2 ubiquitin-conjugating enzymes, in *in vitro* differentiation and cell cycle behavior of HT-29-M6 intestinal mucosecretory cells. *Mol. Cell. Biol.* 18: 576-589.
3. Hofmann, R.M. and Pickart, C.M. 1999. Noncanonical Mms2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. *Cell* 96: 645-653.
4. Deng, L., Wang, C., Spencer, E., Yang, L., Braun, A., You, J., Slaughter, C., Pickart, C. and Chen, Z.J. 2000. Activation of the I κ B complex by TRAF6 requires a dimeric ubiquitin conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103: 351-361.
5. LocusLink Report (LocusID: 602995). <http://www.ncbi.nlm.nih.gov/Locuslink/>

CHROMOSOMAL LOCATION

Genetic locus: UBE2V2 (human) mapping to 8q11.21.

PRODUCT

Mms2 siRNA (h) 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 Mms2 shRNA Plasmid (h): sc-72124-SH and Mms2 shRNA (h) Lentiviral Particles: sc-72124-V as alternate gene silencing products.

For independent verification of Mms2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72124A, sc-72124B and sc-72124C.

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

Mms2 siRNA (h) is recommended for the inhibition of Mms2 expression in human 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

Mms2 (2H11): sc-58391 is recommended as a control antibody for monitoring of Mms2 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Mms2 gene expression knockdown using RT-PCR Primer: Mms2 (h)-PR: sc-72124-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.