

Uev1A siRNA (h): sc-38606

BACKGROUND

Uev1A, also designated Uev1, UBE2V1 for ubiquitin-conjugating enzyme E2 variant 1 and CROC1, shows sequence similarity to other ubiquitin-conjugating enzymes, but lacks the conserved cysteine residue critical for their catalytic activity. 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. The gene encoding Uev1A maps to human chromosome 20q13.13.

REFERENCES

1. Rothofsky, M.L. and Lin, S.L. 1997. CROC1 encodes a protein which mediates transcriptional activation of the human Fos promoter. *Gene* 195: 141-149.
2. Sancho, E., et al. 1998. Role of Uev1, 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., et al. 2000. Activation of the I κ B complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique poly-ubiquitin chain. *Cell* 103: 351-361.
5. Gudgen M, et al. 2004. Interactions within the ubiquitin pathway of *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* 325: 479-486.
6. LocusLink Report (LocusID: 602995). <http://www.ncbi.nlm.nih.gov/Locuslink>.

CHROMOSOMAL LOCATION

Genetic locus: UBE2V1 (human) mapping to 20q13.13.

PRODUCT

Uev1A 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 Uev1A shRNA Plasmid (h): sc-38606-SH and Uev1A shRNA (h) Lentiviral Particles: sc-38606-V as alternate gene silencing products.

For independent verification of Uev1A (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-38606A, sc-38606B and sc-38606C.

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.

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

Uev1A siRNA (h) is recommended for the inhibition of Uev1A 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

Uev1A (H-6): sc-390047 is recommended as a control antibody for monitoring of Uev1A 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 Uev1A gene expression knockdown using RT-PCR Primer: Uev1A (h)-PR: sc-38606-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.