SANTA CRUZ BIOTECHNOLOGY, INC.

UBE4A siRNA (h): sc-63181

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). UBE4A (ubiquitin conjugation factor E4 A), also known as E4, UFD2 or UBOX2, is a 1,066 amino acid protein that functions in the multiubiquitin pathway of protein degradation. Expressed in cortical neurons and in tubular kidney cells, UBE4A is the human homolog of the *Saccharomyces cerevisiae* UFD2 protein and functions with the UBE (ubiquitin enzymes) proteins to catalyze ubiquitin chain assembly. UBE4A may be involved in cell growth and differentiation and can act as an autoantigen in scleroderma, a disease characterized by excessive deposits of collagen in the skin or other organs. Two isoforms of UBE4A exist due to alternative splicing events.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 603753. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Contino, G., et al. 2004. Expression analysis of the gene encoding for the U-box-type ubiquitin ligase UBE4A in human tissues. Gene 328: 69-74.
3. Spinette, S., et al. 2004. UFD2, a novel autoantigen in scleroderma, regulates sister chromatid separation. Cell Cycle 3: 1638-1644.
4. Caren, H., et al. 2006. The two human homologues of yeast UFD2 ubiquitination factor, UBE4A and UBE4B, are located in common neuroblastoma deletion regions and are subject to mutations in tumours. Eur. J. Cancer 42: 381-387.
5. Vazquez-Ortiz, G., et al. 2007. Differentially expressed genes between high-risk human papillomavirus types in human cervical cancer cells. Int. J. Gynecol. Cancer 17: 484-491.
6. Yang, Y., et al. 2007. Differential expression of USP2, USP14 and UBE4A between ovarian serous cystadenocarcinoma and adjacent normal tissues. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 23: 504-506.

CHROMOSOMAL LOCATION

Genetic locus: UBE4A (human) mapping to 11q23.3.

PRODUCT

UBE4A 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBE4A shRNA Plasmid (h): sc-63181-SH and UBE4A shRNA (h) Lentiviral Particles: sc-63181-V as alternate gene silencing products.

For independent verification of UBE4A (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-63181A, sc-63181B and sc-63181C.

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

UBE4A siRNA (h) is recommended for the inhibition of UBE4A 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 µM in 66 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

UBE4A (B-6): sc-365904 is recommended as a control antibody for monitoring of UBE4A 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 UBE4A gene expression knockdown using RT-PCR Primer: UBE4A (h)-PR: sc-63181-PR (20 µl, 585 bp). 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.