SANTA CRUZ BIOTECHNOLOGY, INC.

UBE1L siRNA (m): sc-77414

BACKGROUND

The ubiquitin activating enzyme E1 (UBE1) catalyzes the first step in ubiquitin conjugation to mark cellular proteins for degradation. UBE1 activates ubiquitin by first adenylating (with ATP) its carboxy-terminal glycine residue and thereafter linking this residue to the side chain of a cysteine residue in E1, yielding a ubiquitin-E1 thioester and a free AMP. UBE1 is an example of an X-Y homologous gene, which is X-linked with a distinct Y-linked gene in many mammals. UBE1L (ubiquitin-activating enzyme E1 homolog), also known as UBA7 (ubiquitin-like modifier-activating enzyme 7) or UBE2, is a 1,011 amino acid homolog of UBE1. Like UBE1, UBE1L functions in the activation of ubiquitin through ATP-dependent adenylation. UBE1L is expressed in tumor cells and is a retinoid target that, through conjugation with ISG15 (interferon-induced 15 kDa protein), triggers degradation and apoptosis in acute promyelocytic leukemia.

REFERENCES

1. Kitareewan, S., et al. 2002. UBE1L is a retinoid target that triggers PML/RARα degradation and apoptosis in acute promyelocytic leukemia. Proc. Natl. Acad. Sci. USA 99: 3806-3811.
2. Pitha-Rowe, I., et al. 2004. Involvement of UBE1L in ISG15 conjugation during retinoid-induced differentiation of acute promyelocytic leukemia. J. Biol. Chem. 279: 18178-18187.
3. Pitha-Rowe, I., et al. 2004. Microarray analyses uncover UBE1L as a candidate target gene for lung cancer chemoprevention. Cancer Res. 64: 8109-8115.
4. Zhao, C., et al. 2005. Human ISG15 conjugation targets both IFN-induced and constitutively expressed proteins functioning in diverse cellular pathways. Proc. Natl. Acad. Sci. USA 102: 10200-10205.
5. Krug, R.M., et al. 2005. Properties of the ISG15 E1 enzyme UBE1L. Methods Enzymol. 398: 32-40.
6. Takeuchi, T., et al. 2005. Link between the ubiquitin conjugation system and the ISG15 conjugation system: ISG15 conjugation to the UbcH6 ubiquitin E2 enzyme. J. Biochem. 138: 711-719.

CHROMOSOMAL LOCATION

Genetic locus: Uba7 (mouse) mapping to 9 F2.

PRODUCT

UBE1L 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 UBE1L shRNA Plasmid (m): sc-77414-SH and UBE1L shRNA (m) Lentiviral Particles: sc-77414-V as alternate gene silencing products.

For independent verification of UBE1L (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-77414A, sc-77414B and sc-77414C.

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

UBE1L siRNA (m) is recommended for the inhibition of UBE1L 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 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

UBE1L (B-7): sc-390097 is recommended as a control antibody for monitoring of UBE1L 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 UBE1L gene expression knockdown using RT-PCR Primer: UBE1L (m)-PR: sc-77414-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.