SANTA CRUZ BIOTECHNOLOGY, INC.

UBE1 siRNA (h): sc-61750

BACKGROUND

The ubiquitin activating enzyme E1 (UBE1) catalyzes the first step in ubiquitin conjugation to mark cellular proteins for degradation. Specifically, UBE1 functions to adenylate the C-terminal glycine residue of ubiquitin, a reaction that is ATP-dependent and is proceeded by the formation of a thiolester bond with a cysteine residue of UBE1. The UBE1-activated ubiquitin is then transferred to a ubiquitin conjugated enzyme, which donates the ubiquitin residue to target substrates. The UBE1 gene is an example of an X-Y homologous gene, which is X-linked with a distinct Y-linked gene in many mammals. However, no UBE1 homolog is detectable on the human Y chromosome. UBE1 is thought to escape X inactivation in humans.

REFERENCES

1. Handley, P.M., et al. 1991. Molecular cloning, sequence and tissue distribution of the human ubiquitin-activating enzyme E1. Proc. Natl. Acad. Sci. USA 88: 258-262.
2. Disteche, C.M., et al. 1992. Mapping and expression of the ubiquitin-activating enzyme E1 (UBE1) gene in the mouse. Mamm. Genome 3: 156-161.
3. Coleman, M.P., et al. 1996. A novel gene, DXS8237E, lies within 20 kb upstream of UBE1 in Xp11.23 and has a different X inactivation status. Genomics 31: 135-138.
4. Odorisio, T., et al. 1997. Transcriptional analysis of the candidate spermatogenesis gene Ube1y and of the closely related Ube1x shows that they are coexpressed in spermatogonia and spermatids but are repressed in pachytene spermatocytes. Dev. Biol. 180: 336-343.
5. Carrel, L., et al. 1997. X inactivation analysis and DNA methylation studies of the ubiquitin activating enzyme E1 and PCTAIRE-1 genes in human and mouse. Hum. Mol. Genet. 5: 391-401.
6. Mitchell, M.J., et al. 1998. The origin and loss of the ubiquitin activating enzyme gene on the mammalian Y chromosome. Hum. Mol. Genet. 7: 429-434.

CHROMOSOMAL LOCATION

Genetic locus: UBA1 (human) mapping to Xp11.23.

PRODUCT

UBE1 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 UBE1 shRNA Plasmid (h): sc-61750-SH and UBE1 shRNA (h) Lentiviral Particles: sc-61750-V as alternate gene silencing products.

For independent verification of UBE1 (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-61750A, sc-61750B and sc-61750C.

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 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

UBE1 siRNA (h) is recommended for the inhibition of UBE1 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

UBE1 (2G2): sc-53555 is recommended as a control antibody for monitoring of UBE1 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 UBE1 gene expression knockdown using RT-PCR Primer: UBE1 (h)-PR: sc-61750-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.