

UBE2T siRNA (h): sc-78641

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). UBE2T (ubiquitin-conjugating enzyme E2 T), also known as PIG50 or HSPC150, is a 197 amino acid member of the E2 ubiquitin-conjugating enzyme family. Involved in the protein degradation pathway, UBE2T catalyzes the ATP-dependent attachment of ubiquitin (Ub) to target proteins, thereby tagging them for subsequent destruction by the proteasome. Additionally, UBE2T is thought to be a crucial component of the Faconi anemia pathway of DNA damage repair and, upon self-inactivation, may negatively regulate the Faconi pathway.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 610538. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Andreassen, P.R., et al. 2004. ATR couples FANCD2 monoubiquitination to the DNA-damage response. *Genes Dev.* 18: 1958-1963.
3. Machida, Y.J., et al. 2006. UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. *Mol. Cell* 23: 589-596.
4. Zhang, Y., et al. 2007. Fanconi anemia and ubiquitination. *J. Genet. Genomics* 34: 573-580.

CHROMOSOMAL LOCATION

Genetic locus: UBE2T (human) mapping to 1q32.1.

PRODUCT

UBE2T 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 UBE2T shRNA Plasmid (h): sc-78641-SH and UBE2T shRNA (h) Lentiviral Particles: sc-78641-V as alternate gene silencing products.

For independent verification of UBE2T (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-78641A, sc-78641B and sc-78641C.

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

UBE2T siRNA (h) is recommended for the inhibition of UBE2T 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

UBE2T (4E5): sc-100623 is recommended as a control antibody for monitoring of UBE2T 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 UBE2T gene expression knockdown using RT-PCR Primer: UBE2T (h)-PR: sc-78641-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Liu, L.P., et al. 2017. UBE2T promotes hepatocellular carcinoma cell growth via ubiquitination of p53. *Biochem. Biophys. Res. Commun.* 493: 20-27.
2. Chen, Y., et al. 2021. NEDD4L-induced ubiquitination mediating UBE2T degradation inhibits progression of lung adenocarcinoma via PI3K-AKT signaling. *Cancer Cell Int.* 21: 631.

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.