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New 08/13

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, (Mk)

Description: SignalSilence® UBE2T siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit UBE2T expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

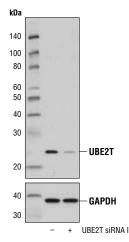
Background: Protein ubiquitination requires the concerted action of the E1, E2 and E3 ubiquitin-conjugating enzymes. Ubiquitin is first activated through ATP-dependent formation of a thiol ester with ubiquitin-activating enzyme E1. The activated ubiquitin is then transferred to a thiol group of ubiquitin-carrier enzyme E2. The final step is the transfer of ubiquitin from E2 to an ε-amino group of the target protein lysine residue, which is mediated by ubiquitin-ligase enzyme E3 (1).

Ubiquitin conjugating-enzyme 2T (UBE2T) is an E2 family member responsible for the ATP-dependent ubiquitin tagging of target proteins for degradation. Research studies indicate that UBE2T plays an important role in the Fanconi anemia pathway and that UBE2T expression is required for normal DNA repair through this pathway. Interaction between UBE2T and FANCL appears to stimulate UBE2T auto monoubiquitination, leading to UBE2T inactivation and negative regulation of the Fanconi anemia pathway (2-4). Additional research details upregulation of UBE2T expression in breast cancer cells and certain lung carcinomas, suggesting a possible involvement in these malignancies (5,6).

Specificity/Sensitivity: SignalSilence® UBE2T siRNA I inhibits human and monkey UBE2T expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® UBE2T siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 μl per well.



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® UBE2T siRNA I (+), using UBE2T (D2L7H) Rabbit mAb #12992 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The UBE2T (D2L7H) Rabbit mAb confirms silencing of UBE2T expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

Entrez-Gene ID #29089 Swiss-Prot Acc. #Q9NPD8

Storage: UBE2T siRNA I is supplied in RNAse-free water. *Aliquot and store at -20°C.*

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

- (1) Hershko, A. (1988) J Biol Chem 263, 15237-40.
- (2) Machida, Y.J. et al. (2006) Mol Cell 23, 589-96.
- (3) Ramaekers, C.H. et al. (2011) Radiother Oncol 101, 190-7.
- (4) Zhang, Y. et al. (2007) J Genet Genomics 34, 573-80.
- (5) Ueki, T. et al. (2009) Cancer Res 69, 8752-60.
- (6) Hao, J. et al. (2008) Tumour Biol 29, 195-203.

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