UBE2T (HSPC150) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Name: HSPC150

Cat. No. **62-0057-020**
Lot. No. **1381**

Quantity: 20 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS - Page 1 of 2

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including regulated and targeted proteosomal degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). UBE2T is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Zhang *et al.* (2000). UBE2T is integral to the Fanconi Anemia (FA) pathway for DNA damage repair. UBE2T binds to the C-terminal PH domain of FANCL the ubiquitin ligase subunit of the FA core complex, which leads to the monoubiquitylation of FANCD2 and FANCI (Longerich *et al.*, 2009; Machida *et al.*, 2006). E3 ligase activity is not determined by assembly of the FA core complex but by the DNA damage-induced subcellular localization of the complex to chromatin. UBE2T and FANCD2 access this subcellular fraction independently and FANCD2 monoubiquitylation is regulated by the formation of an E2/E3 holoenzyme on chromatin. DNA damage in UBE2T-depleted human osteosarcoma cells leads to the formation of abnormal chromosomes that are a hallmark of FA (Alpi *et al.*, 2007). UBE2T expression has been analysed in lung cancer tissue and compared to normal human tissue. UBE2T was found to be significantly upregulated at both the protein and mRNA level suggesting involvement in the malignant cell phenotype (Hao *et al.*, 2008).

References:

Alpi A, Langevin F, Mosedale G, Machida YJ, Dutta A, Patel KJ (2007) UBE2T, the Fanconi anemia core complex, and FANCD2 are recruited independently to chromatin: a basis for the regulation of FANCD2 monoubiquitination. *Mol Cell Biol* **27**, 8421-30.

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Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 20 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~25 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

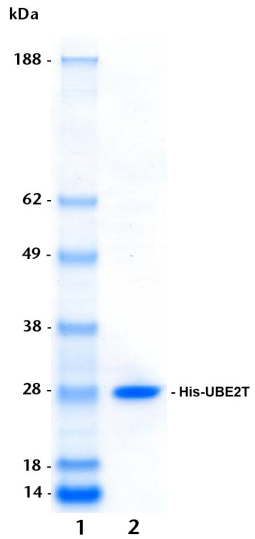
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MGSSHHHHHHSSGLEVLFQGPGS***M***QRASR
LKRELHMLATEPPPGITCWQDKDQMDDLRAQ
ILGGANTPYEKGVFKLEVIIPERYPFEPPQIRFLT
PIYHPNIDSAGRICLDVLKLPPKGAWRPSLNI
ATVLTSIQLLMSEPNPDDPLMADISSEFKYNK
PAFLKNARQWTEKHARQKQKADEEEMLDNL
PEAGDSRVHNSTQKRKASQLVGIEKKFHPDV

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2T (regular text): Start ***bold italics*** (amino acid residues 1-197)
Accession number: NP_054895

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg His-UBE2T



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of His-UBE2T was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2T E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2T enzymes in the presence of ubiquitin and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Sensitivity of the ubiquitin/His-UBE2T thioester bond to the reducing agent DTT was confirmed.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Continued from page 1

Hao J, Xu A, Xie X, Tian T, Gao S, Xiao X, He D (2008) Elevated expression of UBE2T in lung cancer tumors and cell lines. *Tumour Biol* **29**, 195-203.

Longerich S, San Filippo J, Liu D, Sung P (2009) FANCI binds branched DNA and is monoubiquitinated by UBE2T-FANCL. *J Biol Chem* **284**, 23182-6.

Machida YJ, Machida Y, Chen Y, Gurtan AM, Kupfer GM, D'Andrea AD, Dutta A (2006) UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. *Mol Cell* **23**, 589-96.

Zhang QH, Ye M, Wu XY, Ren SX, Zhao M, Zhao CJ, Fu G, Shen Y, Fan HY, Lu G, Zhong M, Xu XR, Han ZG, Zhang JW, Tao J, Huang QH, Zhou J, Hu GX, Gu J, Chen SJ, Chen Z (2000) Cloning and functional analysis of cDNAs with open reading frames for 300 previously undefined genes expressed in CD34+ hematopoietic stem/progenitor cells. *Genome Res* **10**, 1546-60.

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