UBE2T (human; full length), pAb

Alternate Name: HSPC150

Cat. No.	**68-0026-100**	Quantity:	100 µg
Lot. No.	**30263**	Storage:	-20˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including regulated and targeted proteasomal 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 pathway for DNA damage repair. UBE2T binds to the C-terminal PH domain of FANCL the ubiquitin ligase subunit of the Fanconi Anemia (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

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Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: human Ube2T (residues 1-197)

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects Ube2T at ~23kDa

Reactivity: human; other species not tested.

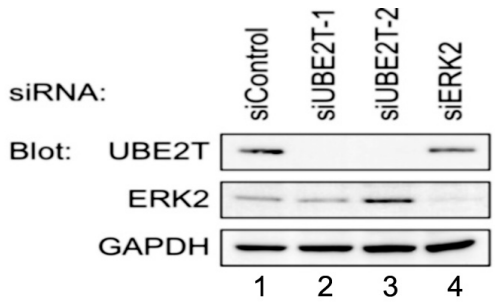
Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
Use 0.1-0.5 µg/ml

Immunoprecipitation:
Use 2 µg/mg cell extract

RNA interference experiment in U2OS cells showing specificity of antibody:



Western Blotting Analysis:
U2OS cells were transfected with either control siRNA, Ube2T-1 siRNA, Ube2T-2 siRNA (lanes 2 and 3) or ERK2 siRNA. By Western blotting the specific recognition of a band corresponding to Ube2T was observed in lysates treated with control siRNA (lane 1) or ERK2 siRNA (lane 4) compared to lysates treated with either Ube2T-1 siRNA or Ube2T-2 siRNA (lanes 2 and 3) where the presence of Ube2T could not be detected when probed with 0.5 µg/ml anti-Ube2T antibody (Cat# 68-0026-100).

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Lot-specific COA version tracker: v1.0.0

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Background

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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).

Antibody Production:
Anti-UBE2T (human) polyclonal antibody was raised in sheep against UBE2T (residues 1-197 of human UBE2T). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-UBE2T pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-UBE2T (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General 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.

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.

Application Reference:

Kelsall IR, Langenick J, MacKay C, Patel KJ and Alpi AF (2012) The Fanconi Anaemia Components UBE2T and FANCM Are Functionally Linked to Nucleotide Excision Repair. *PlosOne* **5**, 36970.

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Lot-specific COA version tracker: v1.0.0