UBE2S (E2-EPF) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: E2-EPF, EC 6.3.2.19, Ubiquitin conjugating enzyme E2-24 kD

Cat. No. **62-0055-020**
Lot. No. **1416**

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). UBE2S is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Liu *et al.* (1992). UBE2S shares 38% identity with yeast Ubc4 and is highly similar to ubiquitin carrier proteins in the core region containing the active-site cysteine. The tumour suppressor protein Von Hippel-Lindau (VHL) forms part of an E3 ligase complex that targets the transcription factor Hypoxia-Inducible Factor-1A (HIF-1A) for degradation. VHL associates with and is targeted by UBE2S for ubiquitin-mediated proteolysis in human cell lines. Over expression of UBE2S increases tumor cell proliferation, invasion, and metastasis through the VHL-HIF pathway and has been found to correlate positively with HIF-1A in tumour cell lines (Jung *et al.*, 2006). An RNAi screen identified UBE2S as an anaphase-promoting complex (APC/C) auxiliary factor that promotes mitotic exit from the spindle-assembly checkpoint (SAC). Knockdown of UBE2S prolongs drug-induced mitotic arrest and suppresses mitotic slippage. UBE2S can also elongate ubiquitin chains initiated by the E2 enzymes UBE2C and UBE2D1, enhancing the degradation of APC/C substrates by the proteasome (Garnett *et al.*, 2009). Recently UBE2S has been shown to assemble K11-specific chains for human and *Drosophila* APC/C. Chain specific activity of UBE2S is dependent on cell cycle-dependent association with the APC/C activators Cdc20 and Cdh1. Depletion of

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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: ~51 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

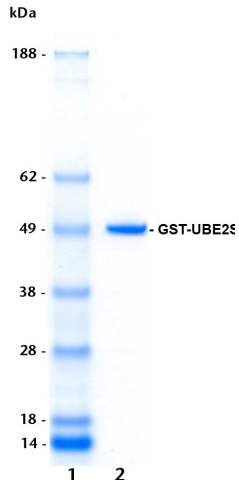
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY EEHLYERDEGDKWRNKKFELGLEFPN LPYYIDGDVKLTQSMAIIRYIADKHNML GGCPKERAEISMLEGAVLDIRYGVSRIAY SKDFETLKVDFLSKLPEMLKMFEDRLCHK TYLNGDHVTHPDFMLYDALDVVLYMDPM CLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAW PLQGWQATFGGGDHPPKSDLEVLFQGPLG S***N***SNVENLPPHIIRLVYKEVTTLTADPPDGIKVF PNEEDLTDLQVTIEGPEGTPYAGGLFRMKLLL GKDFPASPPKGYFLTKIFHPNVGANGEICVNV LKRDWTAELGIRHVLLTIKCLLIHPNPESALNEEA GRLLLENYEEYAARARLLTEIHGGAGGPSGRAEA GRALASGTEASSTDPGAPGGPGGAEGPMAK KHAGERDKKLAAKKKTDKKRALRRL**

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2S (regular text): Start ***bold italics*** (amino acid residues 2-222)
Accession number: AAH65364

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg GST-UBE2S



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2S was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2S E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2S 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/GST-UBE2S thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

UBE2S has been shown to result in severe spindle defects and mitotic delay (Williamson *et al.*, 2009).

References:

Garnett MJ, Mansfeld J, Godwin C, Matsusaka T, Wu J, Russell P, Pines J, Venkitaraman AR (2009) UBE2S elongates ubiquitin chains on APC/C substrates to promote mitotic exit. *Nat Cell Biol* **11**, 1363-9.

Jung CR, Hwang KS, Yoo J, Cho WK, Kim JM, Kim WH, Im DS (2006) E2-EPF UCP targets pVHL for degradation and associates with tumor growth and metastasis. *Nat Med* **12**, 809-16.

Liu Z, Diaz LA, Haas AL, Giudice GJ (1992) cDNA cloning of a novel human ubiquitin carrier protein. An antigenic domain specifically recognized by endemic pemphigus foliaceus autoantibodies is encoded in a secondary reading frame of this human epidermal transcript. *J Biol Chem* **267**, 15829-35.

Williamson A, Wickliffe KE, Mellone BG, Song L, Karpen GH, Rape M (2009) Identification of a physiological E2 module for the human anaphase-promoting complex. *Proc Natl Acad Sci USA* **106**, 18213-8.

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Lot-specific COA version tracker: v1.0.0