

UBE2S (E2-EPF) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

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

Cat. No. 62-0082-020

Lot. No. 1833

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 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). 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 tumour 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

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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: ~27 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

MHHHHHSSGLVPRGSHMASMTGGQOMGRGS
SNVENLPPHIIRLVYKEVTTLTADPPDGIKVF
PNEEDLTDLQVTIEGPEGTPYAGGLFRMKLLL
GKDFPASPPKGYFLTKIFHPNVGANGEICVNV
LKRWDTAELGIRHVLLTIKCLLIHPNPESAL
NEEAGRLLLENYEEYAARARLLTEIHGGAG
GPSGRAEAGRALASGTEASSTDPGAPGGPG
GAEGPMAKKHAGERDKKLAAKKTKDKKRALRRL

Tag (**bold text**): N-terminal His

Protease cleavage site: Thrombin (**LVPR**↓**GS**)

UBE2S (regular text): Start **bold italics** (amino acid residues 2-222)

Accession number: AAH65364

Quality Assurance

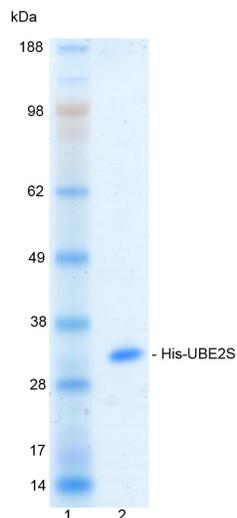
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg His-UBE2S



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2S was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2S E2 enzyme via a transthioesteration reaction. Incubation of the UBE1 and His-UBE2S enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T₀ and T₁₀ minutes. Sensitivity of the ubiquitin/His-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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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 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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