

# UBE2L3 (UbcH7) [6His-tagged]

## E2 – Ubiquitin Conjugating Enzyme

Alternate Names: E2-F1, EC 6.3.2.19, L-UBC, UbcH7, UbcM4, Ubiquitin conjugating enzyme E2-18 kDa UbcH7, Ubiquitin conjugating enzyme UbcH7

**Cat. No.** 62-0040-020  
**Lot. No.** 1376

**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). UBE2L3 is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Moynihan *et al.* (1996). Human UBE2L3 has been mapped to chromosome 22q11.2-q13.1 and shares 97% homology with its mouse homologue (Moynihan *et al.*, 1996; Moynihan *et al.*, 1998). UBE2L3 efficiently mediates the ubiquitylation of E6AP (Nuber *et al.*, 1996). A protein complex comprising UBE2L3, the E3 ligase Parkin and alpha synuclein (alpha-Sp22) has been identified in which the substrate alpha-Sp22 becomes polyubiquitylated in normal human brains and targeted for degradation. Loss of Parkin function causes pathologic accumulation of alpha-Sp22 in the brain which is associated with Parkinson's disease (Shimura *et al.*, 2001). UBE2L3 acts with E6-associated protein (E6-AP) to synergistically enhance the transcriptional activity of the progesterone receptor (PR) and increase its interaction with the steroid receptor coactivator 1 (SRC-1) (Verma *et al.*, 2004). Binding of UBE2L3 to the amino-terminal domain of SMAD 7 stimulates E3 ligase Smurf activity via its HECT domain; recruitment of the complex to the TGFbeta receptor facilitates receptor degradation during TGFbeta signalling (Ogunjimi *et al.*, 2005). Changes in levels of UBE2L3 during the cell cycle regulate entrance into and progression through S phase. UBE2L3 levels decrease during S-phase but are restored in G2, it is thought progression into G2 occurs by

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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:** ~20 kDa

**Purity:** >85% by InstantBlue™ SDS-PAGE

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

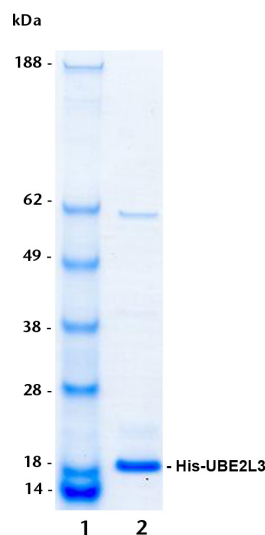
### Protein Sequence:

**MGSSHHHHHSSGLEVLFGQPGSMSAAS**  
RRLMKELEEIRKCGMKNFRNIQVDEAN  
LLTWQGLVDPNPPYDKGAFRIEINFPAYPKPP  
KITFKTKIYHPNIDEKGVCLPVI SAENWKPKTK  
TDQVIQSLIALVNDPQPEHPLRADLAEYSKDRK  
KFCKNAEEFTKKYGEKRPVD

Tag (**bold text**): N-terminal His  
Protease cleavage site: PreScission™ (LEVLFGQ▼GP)  
UBE2L3 (regular text): Start **bold italics** (amino acid residues 1-154)  
Accession number: AAH53368

### Quality Assurance

**Purity:**  
4-12% gradient SDS-PAGE  
InstantBlue™ staining  
lane 1: MW markers  
lane 2: 1 µg His-UBE2L3



### Protein Identification:

Confirmed by mass spectrometry.

### E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2L3 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2L3 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2L3 enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T<sub>0</sub> and T<sub>10</sub> minutes. Sensitivity of the ubiquitin/His-UBE2L3 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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**CERTIFICATE OF ANALYSIS - Page 2 of 2**

## Background

*Continued from page 1*

UBE2L3 modulation of the intra-S phase checkpoint mediated by Chk1 (Whitcomb *et al.*, 2009).

### References:

Moynihan TP, Ardley HC, Leek JP, Thompson J, Brindle NS, Markham AF, Robinson PA (1996) Characterization of a human ubiquitin-conjugating enzyme gene UBE2L3. *Mamm Genome* **7**, 520-5.

Moynihan TP, Cole CG, Dunham I, O'Neil L, Markham AF, Robinson PA (1998) Fine-mapping, genomic organization, and transcript analysis of the human ubiquitin-conjugating enzyme gene UBE2L3. *Genomics* **51**, 124-7.

Nuber U, Schwarz S, Kaiser P, Schneider R, Scheffner M (1996) Cloning of human ubiquitin-conjugating enzymes UbcH6 and UbcH7 (E2-F1) and characterization of their interaction with E6-AP and RSP5. *J Biol Chem* **271**, 2795-800.

Ogunjimi AA, Briant DJ, Pece-Barbara N, Le Roy C, Di Guglielmo GM, Kavsak P, Rasmussen RK, Seet BT, Sicheri F, Wrana JL (2005) Regulation of Smurf2 ubiquitin ligase activity by anchoring the E2 to the HECT domain. *Mol Cell* **19**, 297-308.

Shimura H, Schlossmacher MG, Hattori N, Frosch MP, Trockenbacher A, Schneider R, Mizuno Y, Kosik KS, Selkoe DJ (2001) Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. *Science* **293**, 263-9.

Verma S, Ismail A, Gao X, Fu G, Li X, O'Malley BW, Nawaz Z (2004) The ubiquitin-conjugating enzyme UbcH7 acts as a coactivator for steroid hormone receptors. *Mol Cell Biol* **24**, 8716-26.

Whitcomb EA, Dudek EJ, Liu Q, Taylor A (2009) Novel control of S phase of the cell cycle by ubiquitin-conjugating enzyme H7. *Mol Biol Cell* **20**, 1-9.



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