UBE2L3 (UbcH7) [GST-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. **62-0041-020**
Lot. No. **1399**

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.* (1998). Human UBE2L3 has been mapped to chromosome 22q11.2-q13.1 and shares 97% homology with the 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

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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: ~45 kDa

Purity: >85% by InstantBlue™ SDS-PAGE

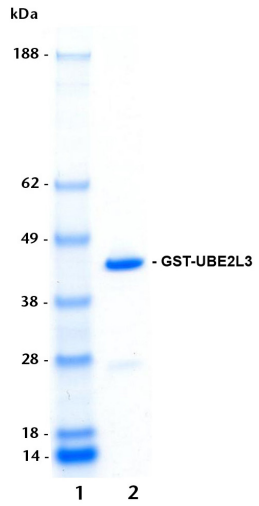
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH LYERDEGDKWRNKKFELGLEFPNLPYYIDGD VKLTQSMAIIRYIADKHNMLGGCPKER AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP DFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATF GGGDHPPKSDLEVLFQGPLGS***M***AASRRLM KELEEIRKCGMKNFRNIQVDEANLLTWQGLIVP DNPPYDKGAFRIEINFPAEYPFKPPKITFKTKIYH PNIDEKGQVCLPVISAENWKPATKTDQVIQS LIALVNDPQPEHPLRADLAEEYSKDRKKFCK NAEEFTKKYGEKRPVD

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼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 GST-UBE2L3



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2L3 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2L3 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2L3 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-UBE2L3 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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Background

Continued from page 1

is thought progression into G2 occurs by 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, et al. (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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