

UBE2G2 (Ubc7) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: EC 6.3.2.19, UBC7, Ubiquitin-conjugating enzyme E2G 2

Cat. No. 62-0029-020
Lot. No. 1395

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). UBE2G2 is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Katsanis and Fischer (1998). The UBE2G2 gene encodes a 165-amino-acid protein that shares 57% sequence identity with UBE2G1. UBE2G2 is involved in protein degradation, including a process known as Endoplasmic Reticulum-Associated Degradation (ERAD). UBE2G2 binds the E3 ligase GP78 and the affinity of this interaction is significantly increased by the G2BR domain on GP78. The UBE2G2/GP78 interaction results in the preassembly of Lys-48-linked ubiquitin chains on the catalytic cysteine of UBE2G2. Growth of the polyubiquitin chain is mediated by an aminolysis-based transfer reaction between two UBE2G2 proteins; a mechanism for transferring preassembled ubiquitin chains from UBE2G2 to the lysine residue in a substrate (Das *et al.*, 2009; Li *et al.*, 2007). The E3 ligase HRD1 interacts with UBE2G2 to form Lys-48-linked polyubiquitin chains on the substrate 3-Hydroxy-3-MethylGlutaryl-coenzyme A Reductase (HMGR) targeting it for degradation (Kikkert *et al.*, 2004). A C4HC3 RING finger-containing ubiquitin ligase of the endoplasmic reticulum - TEB4 - catalyses Lys-48-linked polyubiquitylation employing UBE2G2 *in vitro* (Hassink *et al.*, 2005).

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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: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDG
VKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSIAYSKDFETLKV
FLSKLPEMLKMFEDRLCHKTYLNGDHSVTHP
DFMLYDALDVVLYMDPMLCLDAFPKLVCFK
KRIEAIQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPKSDLEVLFGQPLGSAAGTALKRL
MAEYKQLTLNPPGIVAGPMNEENFFWEAL
IMGPEDTCFEFGVFPAILSFPLDYPLSPPKMRFT
CEMFHPNIYPDGRVCISILHAPGDDPMGYES
SAERWSPVQSVKILLSVVSMLAEPNDESGANV
DASKMWRDDREQFYKIAKQIVQKSLGL

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFGQ▼GP)
UBE2G2 (regular text): Start **bold italics** (amino acid residues 2-165)
Accession number: NP_003334

Quality Assurance

Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg GST-UBE2G2



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of GST-UBE2G2 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2G2 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2G2 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/GST-UBE2G2 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

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References:

Das R, Mariano J, Tsai YC, Kalathur RC, Kostova Z, Li J, Tarasov SG, McFeeters RL, Altieri AS, Ji X, Byrd RA, Weissman AM. (2009) Allosteric activation of E2-RING finger-mediated ubiquitylation by a structurally defined specific E2-binding region of gp78. *Mol Cell* **34**, 674-85.

Hassink G, Kikkert M, Voorden SV, Lee SJ, Spaapen R, Laar TV, Coleman CS, Bartee E, Fruh K, Chau V, Wiertz V. (2005) TEB4 is a C4HC3 RING finger-containing ubiquitin ligase of the endoplasmic reticulum. *Biochem J* **388**, 647-55.

Katsanis N, Fisher EM (1998) Identification, expression, and chromosomal localization of ubiquitin conjugating enzyme 7 (UBE2G2), a human homologue of the *Saccharomyces cerevisiae* *ubc7* gene. *Genomics* **51**, 128-31.

Kikkert M, Doolman R, Dai M, Avner R, Hassink G, Voorden SV, Thanedar S, Roitelman J, Chau V, Wiertz E. (2004) Human HRD1 is an E3 ubiquitin ligase involved in degradation of proteins from the endoplasmic reticulum. *J Biol Chem* **279**, 3525-34.

Li W, Tu D, Brunger AT, Ye Y (2007) A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate. *Nature* **446**, 333-7.



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