

# UBE2D2 (UbcH5b) [GST-tagged]

## E2 – Ubiquitin Conjugating Enzyme

**Alternate Names:** E2(17)KB 2, EC 6.3.2.19, PUBC1, UBC4, UBC4/5 homolog of, UbcH5B, Ubiquitin conjugating enzyme UbcH5B, Ubiquitin-conjugating enzyme E2 D2 transcript variant 1, Ubiquitin-conjugating enzyme E2-17 kDa 2

**Cat. No.** 62-0011-020  
**Lot. No.** 1388

**Quantity:** 20 µg  
**Storage:** -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS

### 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). UBE2D2 is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the human gene was first described by Jensen *et al.* (1995). UBE2D2 shares 95% and 79% sequence identity with the *Drosophila* and *S. cerevisiae* homologues respectively. UBE2D2 can conjugate ubiquitin to targets in an E6AP dependent manner. UBE2D2 forms part of a ubiquitin E3 ligase complex with Cullin1, Skp1, Roc1 and BTRC (Kim *et al.*, 2005). This complex has been shown to mediate activation of NFκB and promote degradation of phosphorylated IκBα. Co-immunoprecipitation has shown that the *Shigella flexneri* effector protein (OspG) interacts with UBE2D2 (Kim *et al.*, 2005). Glial cell missing homolog 1 (GCM1) is an important transcription factor regulating placental cell fusion and it has been shown that UBE2D2 is required for Skp1-Cullin-F-Box (SCF) E3 ligase mediated ubiquitylation of GCM1 (Chiang *et al.*, 2008). UBE2D2 also supports mdm2 mediated ubiquitylation of p53 (Saville *et al.*, 2004).

### References:

Chiang MH, Chen LF, Chen H (2008) Ubiquitin-conjugating enzyme UBE2D2 is responsible for FBXW2 (F-box and WD repeat domain containing 2)-mediated human GCM1 (glial cell missing homolog 1) ubiquitination and degradation. *Biol Reprod* **79**, 914-20.

Jensen JP, Bates PW, Yang M, Vierstra RD, Weissman AM (1995) Identification of a family of closely related human ubiquitin conjugating enzymes. *J Biol Chem* **270**, 30408-14.

Kim DW, Lenzen G, Page AL, Legrain P, Sansonetti PJ, Parsot C (2005) The *Shigella flexneri* effector OspG interferes with innate immune responses by targeting ubiquitin-conjugating enzymes. *Proc Natl Acad Sci USA* **102**, 14046-51.

Saville MK, Sparks A, Xirodimas DP, Wardrop J, Stevenson LF, Bourdon JC, Woods YL, Lane DP (2004) Regulation of p53 by the ubiquitin-conjugating enzymes UbcH5B/C *in vivo*. *J Biol Chem* **279**, 42169-81.

### 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:** ~43 kDa

**Purity:** >98% by InstantBlue™ SDS-PAGE

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

### Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKYEEH  
LYERDEGDKWRNKKFELGLEFPNLPYYIDG  
VKLTQSMAIIRYIADKHNMLGGCPKER  
AEISMLEGAVLDIRYGVSRVIAYSKDFETLKVD  
FLSKLPEMLKMFEDRLCHKTYLNGDHSVTHP  
DFMLYDALDVVLYMDPMCLDAFPKLVCFK  
KRIEAIPIQIDKYLKSSKYIAWPLQGWQATFG  
GGDHPKSDLEVLFGQPLGSALKRIHKELND  
LARDPPAQCSAGPVGDDMFHWQATIMGPND  
SPYQGGVFFLTIHFPTDYPFKPPKVAFTTRIVHPN  
INSNGSICLDILRSQWSPALTISKVLLSICSLC  
PDNDPLVPEIARIYKTDREKYNRIAREWTQKYAM

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

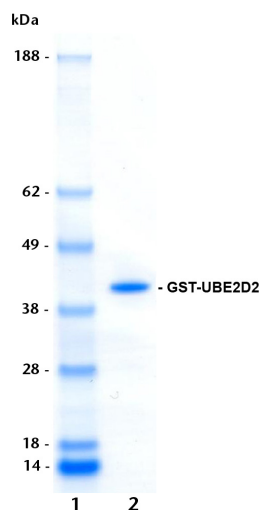
UBE2D2 (regular text): Start **bold italics** (amino acid residues 2-147)

Accession number: NP\_003330

### Quality Assurance

#### Purity:

4-12% gradient SDS-PAGE  
InstantBlue™ staining  
lane 1: MW markers  
lane 2: 1 µg GST-UBE2D2



#### Protein Identification:

Confirmed by mass spectrometry.

#### E2-Ubiquitin Thioester Loading Assay:

The activity of GST-UBE2D2 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2D2 E2 enzyme via a transthioester reaction. Incubation of the UBE1 and GST-UBE2D2 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/GST-UBE2D2 thioester bond to the reducing agent DTT was confirmed.



www.ubiquigent.com  
Dundee, Scotland, UK

#### ORDERS / SALES SUPPORT

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**US Toll-Free:** 1-888-4E1E2E3 (1-888-431-3233)  
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**International:** +44 (0) 1382 381147 (9AM-5PM UTC)  
**US/Canada:** +1-617-245-0020 (9AM-5PM UTC)  
**Email:** tech.support@ubiquigent.com

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Lot-specific COA version tracker: v1.0.0