

UBE2D2 (UbcH5b) [6His-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. 62-0073-020

Lot. No. 1824

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). 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).

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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: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

M H H H H H S S G L V P R G S H M A S M T G
G Q Q M G R G S A L K R I H K E L N D L A R D P
P A Q C S A G P V G D D M F H W Q A T I M G P N D
S P Y Q G G V F F L T I H F P T D Y P F K P P
K V A F T T R I Y H P N I N S N G S I C L D I L R
S Q W S P A L T I S K V L L S I C S L L C D P N P
D D P L V P E I A R I Y K T D R E K Y N R I A R E
W T Q K Y A M

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

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

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

Accession number: NP_003330

Quality Assurance

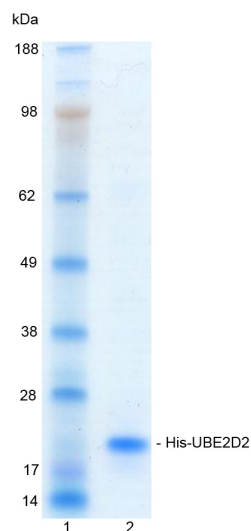
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg His-UBE2D2



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2D2 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2D2 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2D2 enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T₀ and T₁₀ minutes. The sensitivity of this ubiquitin/His-UBE2D2 thioester bond to the reducing agent DTT was demonstrated.



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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:

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.



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