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. **62-0011-100**
Lot. No. **1388**

Quantity: 100 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: 100 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

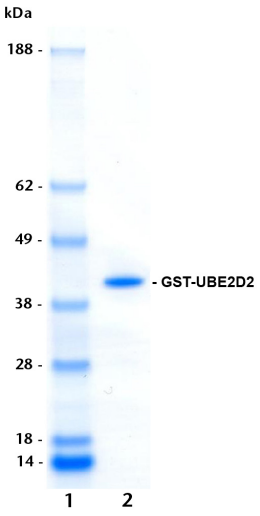
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD
FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP
DFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLFQGPLGS***A***LKRIHKELND**
LARDPPAQCSAGPVGDDMFHWQATIMGPND
SPYQGGVFFLTIHFPTDYPFKPPKVAFTTRIYHPN
INSNGSICLDILRSQWSPALTISKVLLSICSLLCDP
NPDDPLVPEIARIYKTDREKYNRIAREWTQKYAM

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼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 transthiolation 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_0 and T_{10} 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
International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0