

UBE2D3 (UbcH5c) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: E2(17)KB 3, EC 6.3.2.19, MGC43926, MGC5416, UBC4/5, UbcH5C, Ubiquitin carrier protein D3, Ubiquitin conjugating enzyme E2-17 kDa 3

Cat. No. **62-0013-100**
Lot. No. **1389**

Quantity: 100 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 the 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). UBE2D3 is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the gene was first described by Jensen *et al.* (1995). Human UBE2D3 shares 94% and 79% sequence identity with the *Drosophila* and *S. cerevisiae* homologues respectively. UBE2D3 can conjugate ubiquitin to targets such as p53 in an E6AP dependent manner (Jensen *et al.*, 1995). Upregulation of UBE2D3 following treatment with Retinoic Acid (RA) has been shown to induce differentiation and growth arrest in NB4 human promyelocytic cells. UBE2D3 associates with Cyclin D1 and mediates retinoic acid induced cyclin D1 degradation (Hattori *et al.*, 2007). Activation of the IKK complex is mediated by unanchored polyubiquitin chains formed by UBE2D3 and TRAF6 (Xia *et al.*, 2009). Zipper-interacting protein kinase (ZIPK) is a serine/threonine kinase implicated in cell death and transcriptional regulation. UBE2D3 induces ZIPK accumulation in promyelocytic leukemia protein nuclear bodies resulting in their ubiquitylation

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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

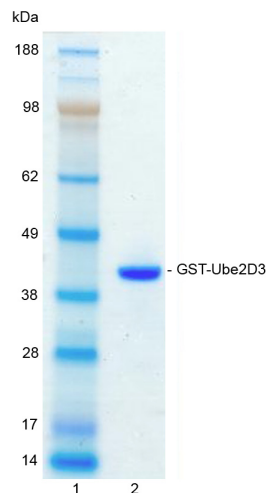
Protein Sequence:

**MSPILGYWKIKGLVQPTRLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSR IAYS KDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHSVTHPD
FMLYDALDVVL YMDP MCLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLVFOGPLGSALKRINKELSD
LARDPPAQC SAGPVGDDMFHWQATIMGPN
SPYQGGVFFLT IHFPTDYPFKPPKVAFTTRI
YHPNINSNGSICLDILRSQWSPALTISKVLL
SICSLLCDPNPDDPLVPEIARIYKTRDRKYN
RISREWTQKYAM**

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLVFOG↓GP)
UBE2D3 (regular text): Start **bold italics** (amino acid residues 2-147)
Accession number: NP_003331

Quality Assurance

Purity: 4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-UBE2D3



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of GST-UBE2D3 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2D3 E2 enzyme via a transthioylation reaction. Incubation of the UBE1 and GST-UBE2D3 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-UBE2D3 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

Continued from page 1

(Ohbayashi *et al.*, 2008). Meibomian cell carcinoma (MCC) is a malignant tumour of the meibomian glands located in the eyelids, UBE2D3 has been identified by RT/PCR as one of five genes found to be upregulated in MCC tumours (Kumar *et al.*, 2007).

References:

Hattori H, Zhang X, Jia Y, Subramanian KK, Jo H, Loison F, Newburger PE, Luo HR (2007) RNAi screen identifies UBE2D3 as a mediator of all-trans retinoic acid-induced cell growth arrest in human acute promyelocytic NB4 cells. *Blood* **110**, 640-50.

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.

Kumar A, Kumar Dorairaj S, Prabhakaran VC, Prakash DR, Chakraborty S (2007) Identification of genes associated with tumorigenesis of meibomian cell carcinoma by microarray analysis. *Genomics* **90**, 559-66.

Ohbayashi N, Okada K, et al. (2008) Physical and functional interactions between ZIP kinase and UbcH5. *Biochem Biophys Res Commun* **372**, 708-12.

Xia ZP, Sun L, Chen X, Pineda G, Jiang X, Adhikari A, Zeng W, Chen ZJ (2009) Direct activation of protein kinases by unanchored polyubiquitin chains. *Nature* **461**, 114-9.



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