# **UBE2D3** (UbcH5c) [6His-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.** **62-0074-020**
**Lot. No.** **1825**

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 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. The E3 ligase E6AP mediates the conjugation of ubiquitin to targets such as p53 via UBE2D3 (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 also 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 leukaemia protein nuclear bodies resulting in their ubiquitylation (Ohbayashi *et al.*, 2008). Meibomian Cell Carcinoma (MCC) is

*Continued on page 2*

## 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:** ~19 kDa

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

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

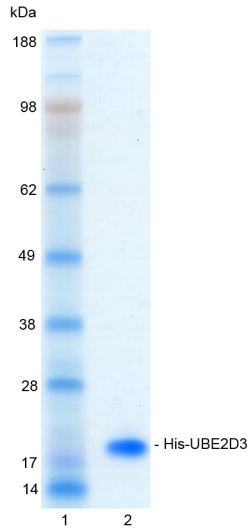
**Protein Sequence:**

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

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼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 His-UBE2D3




**Protein Identification:**
Confirmed by mass spectrometry.

**E2-Ubiquitin Thioester Loading Assay:**
The activity of His-UBE2D3 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2D3 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2D3 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/His-UBE2D3 thioester bond to the reducing agent DTT was confirmed.

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



**Lot-specific COA version tracker:** v1.0.0

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## Background

*Continued from page 1*

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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