# UBE2D4 (UbcH5d) [untagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Name: LOC51619 protein, UbcH5d

Cat. No.	62-0017-100
Lot. No.	1461

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 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). UBE2D4 is a member of the E2 ubiquitin-conjugating enzyme family and the human gene was first described by Colland *et al.* (2004).

#### **References:**

Colland F, Jacq X, Trouplin V, Mougin C, Groizeleau C, Hamburger A, Meil A, Wojcik A, Legrain P, Gauthier J (2004) Functional proteomics mapping of a human signaling pathway. *Genome Res* **14**, 1324-32.



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: ~20 kDa

Purity: >75% by InstantBlue™ SDS-PAGE

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

## Quality Assurance

#### **Purity:**

4-12% gradient SDS-PAGE InstantBlue™ staining Lane 1: MW markers Lane 2: 1 μg UBE2D4



#### **Protein Sequence:**

GSHMASMTGGQQMGRGSEFELGSTSNGRQCA GIRPCAAAMALKRIQKELTDLQRDPPAQCSAG PVGDDLFHWQATIMGPNDSPYQGGVFFLTI HFPTDYPFKPPKVAFTTKIYHPNINSNGSI CLDILRSQWSPALTVSKVLLSICSLLCDPNPD DPLVPEIAHTYKADREKYNRLAREWTQKYAM

The residues <u>underlined</u> remain after cleavage and removal of the purification tag. UBE2D4 (regular text): Start **bold italics** (amino acid residues 1-147) Accession number: NP\_057067

# Protein Identification:

Confirmed by mass spectrometry.

#### E2-Ubiquitin Thioester Loading Assay:

The activity of UBE2D4 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the UBE2D4 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and UBE2D4 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/UBE2D4 thioester bond to the reducing agent DTT was confirmed.



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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 UIC) Email: tech.support@ubiquigent.com

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