# **UBE2D4** (UbcH5d) [untagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Name: LOC51619 protein, UbcH5d

Cat. No. 62-0017-020

Lot. No. 1461

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.

## **Physical Characteristics**

20 µg

-70°C

Species: human

Quantity:

Storage:

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: >75% by InstantBlue™ SDS-PAGE

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

aliquot as required

### **Protein Sequence:**

GSHMASMTGGQQMGRGSEFELGSTSNGRQCA GIRPCAAAMALKRIQKELTDLQRDPPAQCSAG PVGDDLFHWQATIMGPNDSPYQGGVFFLTI **HFPTDYPFKPPKVAFTTKIYHPNINSNGSI** CLDILRSQWSPALTVSKVLLSICSLLCDPNPD DPLVPEIAHTYKADREKYNRLAREWTQKYAM

The residues underlined remain after cleavage and removal of the purification tag

UBE2D4 (regular text): Start bold italics (amino acid

residues 1-147)

Accession number: NP\_057067

## Quality Assurance

### **Purity:**

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



### **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<sub>0</sub> and T<sub>10</sub> minutes. Sensitivity of the ubiquitin/UBE2D4 thioester bond to the reducing agent DTT was confirmed.



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