UBE2D4 (UbcH5d) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Name: LOC51619 protein, UbcH5d

Cat. No. 62-0015-020 Lot. No. 30192

FOR RESEARCH USE ONLY

Quantity: 20 μg Storage: -70°C

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

Reference:

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

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: ~22 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

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

aliquot as required

Protein Sequence:

MGSSHHHHHHSSGLVPRGSHMASMTG GQQMGRGSEFELGSTSNGRQCAGIRPCAAA MALKRIQKELTDLQRDPPAQCSAGPVGDDLF HWQATIMGPNDSPYQGGVFFLTIHFPTDYP FKPPKVAFTTKIYHPNINSNGSICLDILRSQWS PALTVSKVLLSICSLLCDPNPDDPLVPEIAHTYKA DREKYNRLAREWTQKYAM

Tag (bold text): N-terminal His

Protease cleavage site: Thrombin (<u>LVPR▼GS</u>)
UBE2D4 (regular text): Start **bold italics** (amino acid

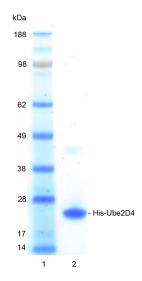
residues 1-147)

Accession number: NP_057067

Quality Assurance

Purity:

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



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

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



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