UBE2D4 (UbcH5d) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Name: LOC51619 protein, UbcH5d

Cat. No. **62-0016-020**
Lot. No. **1390**

Quantity: 20 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 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: ~44 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

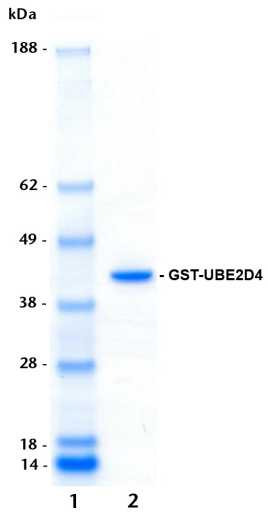
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH LYERDEGDKWRNKKFELGLEFPNLPYYIDGD VKLTQSMAIIRYIADKHNMLGGCPKER AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP DFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG GGDHPPKSDLEVLFQGPLGSPGIPGSTRAAA ***M***ALKRIQKELTDLQRDPPAQCSAGPVGDDLF HWQATIMGPNDSPYQGGVFFLTIHFPTDYP FKPPKVAFTTKIYHPNINSNGSICLDILRSQWS PALTVSKVLLSICSLLCDPNPDDPLVPEIAHTYKA DREKYNRLAREWTQKYAM

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
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 GST-UBE2D4



Protein Identification:
Confirmed by mass spectrometry.

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

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Lot-specific COA version tracker: v1.0.0