UBE2D1 (UbcH5a) [untagged]
E2 – Ubiquitin Conjugating Enzyme

Alternate Names: E2(17)KB 1, EC 6.3.2.19, SFT, Stimulator of Fe transport, homolog of UBC4/5, UbcH5, UbcH5A. Ubiquitin protein ligase, Ubiquitin-conjugating enzyme E2-17 kDa 1, Ubiquitin-conjugating enzyme UbcH5A

Cat. No. 62-0010-100
Lot. No. 1458

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NOT FOR USE IN HUMANS

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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). UBE2D1 is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the human gene was first described by Scheffner et al. (1994). UBE2D1 shares 89% sequence identity with its Drosophila homologue and mediates E6/UBE3A (E6AP)-induced ubiquitylation of p53 (Jensen et al., 1995; Scheffner et al., 1994). Ubiquitylation of the yeast PTS1 import receptor (pex5p) has been demonstrated in an in vitro assay in the presence of the human UBE2D1 in combination with the ring domain of the yeast E3 ligase pex10p (Williams et al., 2008). Sequence encoding the stimulated Iron transport gene SFT overlaps with intron 7 and exon 6 of UBE2D1, and RT/PCR has shown significantly upregulated levels of UBE2D1 in livers of iron-overloaded patients with hereditary hemochromatosis (Gehrke et al., 2003).

Physical Characteristics

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: ~18 kDa
Purity: >98% 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 UBE2D1

Protein Sequence:

GSHMASMTGGOMGRGSAKRLKQKELSDDLQRD
PPAHCSAPGDGLFHWQATIMGPDASYQG
GVFFTVEHPFTYPFPKPKTAPFTKIIHNPIN
SNGSICLDILRSGWAPALTVSKVLSSICSL
CDPNDPLVDPDIAYKSDKEKYNHRME
WTQKYAM

The residues underlined remain after cleavage and removal of the purification tag. UBE2D1 (regular text): Start bold italics (amino acid residues 2-147)
Accession number: NP_003329

Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of UBE2D1 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the UBE2D1 E2 enzyme via a transthioleation reaction. Incubation of the UBE1 and UBE2D1 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/UBE2D1 thioester bond to the reducing agent DTT was confirmed.

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References:


