

UBE2D1 (UbcH5a) [GST-tagged]

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-conjugating enzyme E2-17 kDa 1, Ubiquitin-conjugating enzyme UbcH5A

Cat. No. 62-0009-020
Lot. No. 1387

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). 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).

References:

Gehrke SG, Riedel HD, Herrmann T, Hadaschik B, Bents K, Veltkamp C, Stremmel W (2003) UbcH5A, a member of human E2 ubiquitin-conjugating enzymes, is closely related to SFT, a stimulator of iron transport, and is up-regulated in hereditary hemochromatosis. *Blood* **101**, 3288-93.

Jensen JP, Bates PW, Yang M, Vierstra RD, Weissman AM (1995) Identification of a family of closely related human ubiquitin conjugating enzymes. *J Biol Chem* **270**, 30408-14.

Scheffner M, Huibregtse JM, Howley PM (1994) Identification of a human ubiquitin-conjugating enzyme that mediates the E6-AP-dependent ubiquitination of p53. *Proc Natl Acad Sci USA* **91**, 8797-801.

Williams C, van den Berg M, Geers E, Distel B (2008) Pex10p functions as an E3 ligase for the Ubc4p-dependent ubiquitination of Pex5p. *Biochem Biophys Res Commun* **374**, 620-4.

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: ~43 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

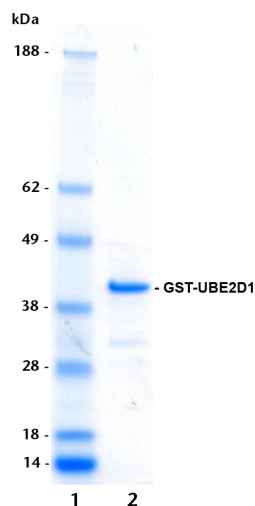
MSPILGYWKIKGLVQPTRLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDG
VKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSRAYSKDFETLKVD
FLSKLPEMLKMFEDRLCHKTYLNGDHTHP
DFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPIQIDKYLKSSKYIAWPLQGQWQATF
GGGDHPPKSDLEVLFGQPLGSAKRIQKELS
DLQRDPPAHCSAGPVGDDLFHWQATIMGPPD
SAYQGGVFFLTVHFPTDYPFKPPKIAFTTKIYHP
NINSNGSICLDILRSQWSPALTVSKVLLSICSL
CDPNPDDPLVPDIAQIYKSDKEKYNRHARE
WTQKYAM

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFGQ▼GP)
UBE2D1 (regular text): Start **bold italics** (amino acid residues 2-147)
Accession number: NP_003329

Quality Assurance

Purity:

4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg GST-UBE2D1



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

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



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Lot-specific COA version tracker: v1.0.0