# 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**

Quantity: 100 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

**CERTIFICATE OF ANALYSIS Page 1 of 2**

## 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).

*Continued on page 2*

## 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

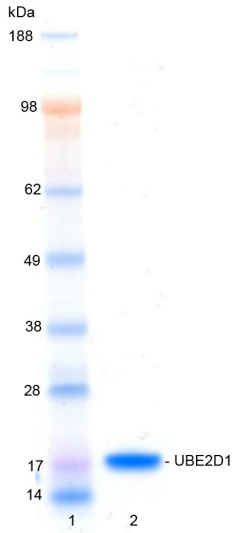
**Protein Sequence:**

GSHMASMTGGQQMGRGS***A***LKRIQKELSDLQRD
PPAHCSAGPVGDDLFHWQATIMGPPDSAYQG
GVFFLTVHFPTDYPFKPPKIAFTTKIYHPNIN
SNGSICLDILRSQWSPALTVSKVLLSICSLL
CDPNPDDPLVPDIAQIYKSDKEKYNRHARE
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

## Quality Assurance

**Purity:**
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg UBE2D1




**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 transthiolation reaction. Incubation of the UBE1 and UBE2D1 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/UBE2D1 thioester bond to the reducing agent DTT was confirmed.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.



**Lot-specific COA version tracker:** v1.0.0

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## Background

*Continued from page 1*

### 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.

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