UBE2Z (USE1) [6His-tagged]

E2 - FAT10 or Ubiquitin Conjugating Enzyme

Alternate Names: UBA6-Specific E2; USE1

Cat. No.	**62-0086-020**	Quantity:	20 µg
Lot. No.	**1837**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 1

Background

The enzymes of the FATylation pathway play a pivotal role in a number of cellular processes including regulated and targeted proteasomal degradation of ubiquitylated substrate proteins (Buchsbaum *et al.*, 2011). Three classes of enzymes are involved in the process of FATylation; the activating enzyme Uba6, conjugating enzymes (E2s) and protein ligases (E3s). UBE2Z is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Gu *et al.* (2007) and Jin *et al.* (2007). UBE2Z is widely expressed in human tissues and expression is particularly high in the placenta, pancreas, spleen and testis (Gu *et al.*, 2007). UBE2Z has been identified as an interaction partner of FAT10. FAT10 can be transferred from Uba6 to UBE2Z *in vitro* and both FAT10 and UBE2Z have been co-immunoprecipitated from intact cells. Down regulation of UBE2Z by siRNA resulted in a strong reduction of endogenous conjugate formation suggesting UBE2Z is the major E2 conjugating enzyme in the FAT10 cascade (Aichem *et al.*, 2010).

References:

Aichem, A., C. Pelzer, *et al.* (2010) USE1 is a bispecific conjugating enzyme for ubiquitin and FAT10, which FAT10ylates itself in cis. *Nat Commun* **1**: 13.

Buchsbaum, S., B. Bercovich, *et al.* (2011) FAT10 is a proteasomal degradation signal which is itself regulated by ubiquitination. *Mol Biol Cell.* (in press)

Gu, X., F. Zhao, *et al.* (2007) Cloning and characterization of a gene encoding the human putative ubiquitin conjugating enzyme E2Z (UBE2Z). *Mol Biol Rep* **34**(3): 183-8.

Jin, J., X. Li, *et al.* (2007) Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* **447**(7148): 1135-8.

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: ~40.6 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

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

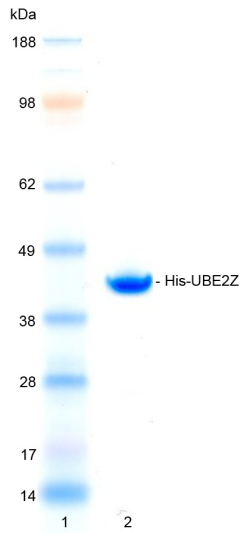
Protein Sequence:

MGSSHHHHHHSSGLEVLFQGPGS***M***AESPTEE
AATAGAGAAGPGASSVAGVVGVSGSGGGFGP
PFLPDVWAAAAAAGGAGGPGSGLAPLPGLPP
SAAAHGAALLSHWDPTLSSDWDGERTAPQCLL
RIKRDIMSIYKEPPPGMFVVPDTVDMTKIHALIT
GPFDTPYEGGFFLFVFRCPPDYPIHPPRVKLMTT
GNNTVRFNPNFYRNGKVCLSILGTWTGPAWSPAQ
SISSVLISIQSLMTENPYHNEPGFEQERHPGD
SKNYNECIRHETIRVAVCDMMEGKCPCPEPL
RGVMEKSFLEYYDFYEVACKDRLHLQGQTMQDPF
GEKRGHFDYQSLLMRLGLIRQKVLERLHNENAE
MDSDSSSSGTETDLHGSLRV

Tag (**bold text**): N-terminal His
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2Z (regular text): Start ***bold italics*** (amino acid residues 1-354)
Accession number: NP_075567.2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg His-UBE2Z



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of His-UBE2Z was validated by loading E1 Uba6 activated ubiquitin onto the active cysteine of the His-UBE2Z E2 enzyme via a transthiolation reaction. Incubation of the Uba6 and His-UBE2Z enzymes in the presence of ubiquitin and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Under these conditions tested no His-UBE2Z/ubiquitin thioester loading was observed.

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Lot-specific COA version tracker: v1.0.0