UBE2Z (USE1) [GST-tagged]

E2 - FAT10 or Ubiquitin Conjugating Enzyme

Alternate Names: UBA6-Specific E2; USE1

Cat. No. 62-0060-020

Lot. No. 1418

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

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

20 µg

-70°C

Species: human

Quantity:

Storage:

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: ~65 kDa

Purity: >85% by InstantBlue™ SDS-PAGE

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

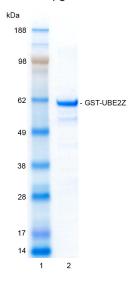
aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:

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



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of GST-UBE2Z was validated by loading E1 Uba6 activated ubiquitin onto the active cysteine of the GST-UBE2Z E2 enzyme via a transthiolation reaction. Incubation of the Uba6 and GST-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 GST-UBE2Z/ubiquitin thioester loading was observed.



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH LYERDEGDKWRNKKFELGLEFPNLPYYIDGD **VKLTQSMAIIRYIADKHNMLGGCPKERAEISM** LEGAVLDIRYGVSRIAYSKDFETLKVDFL SKLPEMLKMFEDRLCHKTYLNGDHVTHPD FMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG GGDHPPKSDLEVLFQGPLGSMAESPTEEAATA GAGAAGPGASSVAGVVGVSGSGGGFGPPFLPD VWAAAAAGGAGGPGSGLAPLPGLPPSAAAH GAALLSHWDPTLSSDWDGERTAPQCLLRIKRDIM SIYKEPPPGMFVVPDTVDMTKIHALITGPFDT PYEGGFFLFVFRCPPDYPIHPPRVKLMTTGNNT VRFNPNFYRNGKVCLSILGTWTGPAWSPAQ SISSVLISIQSLMTENPYHNEPGFEQERHPGD SKNYNECIRHETIRVAVCDMMEGKCPCPEPL RGVMEKSFLEYYDFYEVACKDRLHLOGOTMODPF GEKRGHFDYQSLLMRLGLIRQKVLERLHNENAE MDSDSSSSGTETDLHGSLRV

Tag (bold text): N-terminal GST

Protease cleavage site: PreScission™ (<u>LEVLFQ▼GP</u>)
UBE2Z (regular text): Start **bold italics** (amino acid residues

1-354)

Accession number: NP_075567.2



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