UBE2E1 (UbcH6) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: UbcH6, UbcH6, Ubiquitin conjugating enzyme UbcH6

Cat. No. **62-0018-020**
Lot. No. **1391**

Quantity: 20 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 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). UBE2E1 is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the human gene was first described by Nuber *et al.* (1996). UBE2E1 shares 74% sequence homology with UBE2D1 and has an N-terminal extension of approximately 40 amino acids. A tumour suppressor candidate – tumour-suppressing subchromosomal transferable fragment cDNA (TSSC5) – is located in the region of human chromosome 11p15.5 linked with Beckwith-Wiedemann syndrome and associated with susceptibility to Wilms tumor (Yamada and Gorbsky, 2006). UBE2E1 functions in concert with a novel ubiquitin ligase RING-finger protein 105 (RING105) to ubiquitylate TSSC5. Regulation of TSSC5 function mediated via UBE2E1 and RING105 could define a novel ubiquitin proteosome pathway. The E3 ligase Ro52 mediates ubiquitylation of its substrates through UBE2E1 in the nucleus and translocation of this E3 ligase to the nucleus is dependent on amino acids 381-470 of the B30.2 region (Espinosa *et al.*, 2008). UBE2E1 also modulates the transcriptional repression activity of Ataxin-1, the gene product of Spinocerebellar ataxia type 1 (SCA1). SCA1 is an autosomal-dominant neurodegenerative disorder characterized by ataxia and progressive motor deterioration, which is caused by expansion of the polyglutamine tract in Ataxin-1. Ataxin-1 binds with UBE2E1 through its AXH domain and *in vitro* the rate of Ataxin-1 degradation is regulated by UBE2E1. UBE2E1

Continued on page 2

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: ~49 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

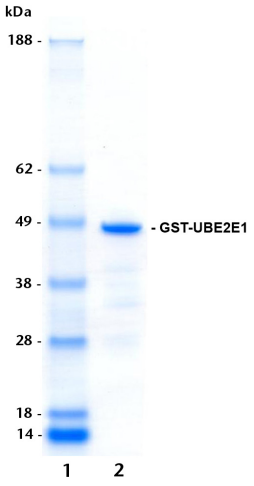
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD
FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP
DFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKSDLEVLFQGPLGSPGIPG
STRAAA***M***SDDDSRASTSSSSSSSNQQTEKET
NTPKKKESKVSMSKNSKLLSTSAKRIQKELA
DITLDPPPNCSAGPKGDNIYEWRSTILGPPGS
VYEGGVFFLDITFTPEYPFKPPKVTFRTRIYHCNIN
SQGVICLDILKDNWSPALTISKVLLSICSLLTDCN
PADPLVGSIATQYMTNRAEHDRMARQWTKRYAT

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2E1 (regular text): Start ***bold italics*** (amino acid residues 1-193)
Accession number: AAH09139

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg GST-UBE2E1



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2E1 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2E1 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2E1 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/GST-UBE2E1 thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

may have some therapeutic potential in the treatment of SCA1 by modulating the degradation of Ataxin-1 (Hong *et al.*, 2008; Lee *et al.*, 2008).

References:

Espinosa A, Oke V, Elfving A, Nyberg F, Covacu R, Wahren-Herlenius M (2008) The autoantigen Ro52 is an E3 ligase resident in the cytoplasm but enters the nucleus upon cellular exposure to nitric oxide. *Exp Cell Res* **314**, 3605-13.

Hong S, Lee S, Cho SG, Kang S (2008) UbcH6 interacts with and ubiquitinates the SCA1 gene product ataxin-1. *Biochem Biophys Res Commun* **371**, 256-60.

Lee S, Hong S, Kang S (2008) The ubiquitin-conjugating enzyme UbcH6 regulates the transcriptional repression activity of the SCA1 gene product ataxin-1. *Biochem Biophys Res Commun* **372**, 735-40.

Nuber U, Schwarz S, Kaiser P, Schneider R, Scheffner M (1996) Cloning of human ubiquitin-conjugating enzymes UbcH6 and UbcH7 (E2-F1) and characterization of their interaction with E6-AP and RSP5. *J Biol Chem* **271**, 2795-800.

Yamada HY, Gorbsky GJ (2006) Tumor suppressor candidate TSSC5 is regulated by UbcH6 and a novel ubiquitin ligase RING105. *Oncogene* **25**, 1330-9.

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