

UBE2E1 (UbcH6) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: UbcH6, UbcH6, Ubiquitin conjugating enzyme UbcH6

Cat. No. 62-0075-100

Lot. No. 1826

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). 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 contains an N-terminal extension of approximately 40 amino acids. A tumour suppressor candidate, tumour-suppressing sub-chromosomal 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 proteasome 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 A-

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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: ~26 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

M G S S H H H H H S S G LEVLFQGPSPPEF
PGVDSKAAAMSDDDSRASTSSSSSSSS
NQQTEKETNTPKKKESKVSMSKN
SKLLSTSAKRIQKELADITLDPNPNC
SAGPKGDNIYEWIRSTILGPPGSVYEG
GVFFLDITFTPEYFPKPPKVTFRTRI
YHCNINSQGVICLDILKDNWSPAL
TISKVLLSICSLLTDCNPADPLVGS
IATQYMTNRAEHDRMARQWTKRYAT

Tag (**bold text**): N-terminal His

Protease cleavage site: PreScission™ (LEVLFQ▼GP)

UBE2E1 (regular text): Start **bold italics** (amino acid residues 1-193)

Accession number: AAH09139

Quality Assurance

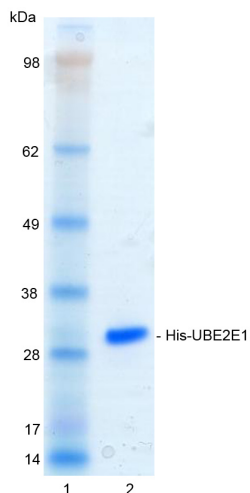
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg His-UBE2E1



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2E1 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2E1 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2E1 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/His-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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taxin-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 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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