

UBE2E3 (UbcH9) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: UbcH9, UbcM2, Ubiquitin conjugating enzyme UbcH9

Cat. No. 62-0076-020

Lot. No. 1827

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 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). UBE2E3 is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the gene was first described by Ito *et al.* (1999). UBE2E3 binds to the RING-finger proteins ARA54 and RNF8, thought to act as E3 ligases in the ubiquitylation of nuclear proteins (Ito *et al.*, 2001). The epithelial Na⁺ channel (ENaC) is regulated by UBE2E3 and the E3 ligase NEDD4.2. UBE2E3 interacts with NEDD4.2 via its UBC domain and ubiquitylation of ENaC occurs by NEDD4.2 binding the PY motifs of its α, β and γ subunits (Debonneville and Staub, 2004). NEDD4.2 is a negative regulator of ENaC and deletions in the PY motifs of the β and γ subunits of ENaC cause Liddle's syndrome, an inherited form of hypertension. The loss of NEDD4.2 binding sites in mutated ENaC causes an increase in channel number at the cell surface and increased Na⁺ reabsorption by the distal nephron, resulting in hypertension (Abriel *et al.*, 1999).

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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: ~26 kDa

Purity: >90% 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 L V P R G S H M A S M T
G G Q Q M G R R S M S S D R Q R S D D E S P S T
S S G S S D A D Q R D P A A P E P E E Q E E R K P
S A T Q Q K K N T K L S S K T T A K L S T
S A K R I Q K E L A E I T L D P P P N C S A G P K
G D N I Y E W R S T I L G P P G S V Y E G G V F
F L D I T F S S D Y P F K P P K V T F R T R I Y H C
N I N S Q G V I C L D I L K D N W S P A L T I S K V
L L S I C S L L T D C N P A D P L V G S I A T Q Y L T
N R A E H D R I A R Q W T K R Y A T

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

Protease cleavage site: Thrombin (LVPR▼GS)

UBE2E3 (regular text): Start **bold italics** (amino acid residues 1-207)

Accession number: NP_006348

Quality Assurance

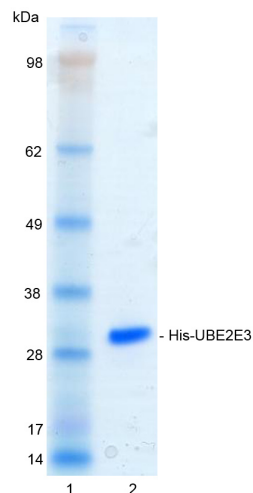
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg His-UBE2E3



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2E3 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2E3 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2E3 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-UBE2E3 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

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References:

Abriel H, Loffing J, Rebhun JF, Pratt JH, Schild L, Horisberger JD, Rotin D, Staub O (1999) Defective regulation of the epithelial Na⁺ channel by Nedd4 in Liddle's syndrome. *J Clin Invest* **103**, 667-73.

Debonneville C, Staub O (2004) Participation of the ubiquitin-conjugating enzyme UBE2E3 in Nedd4-2-dependent regulation of the epithelial Na⁺ channel. *Mol Cell Biol* **24**, 2397-409.

Ito K, Adachi S, Iwakami R, Yasuda H, Muto Y, Seki N, Okano Y (2001) N-Terminally extended human ubiquitin-conjugating enzymes (E2s) mediate the ubiquitination of RING-finger proteins, ARA54 and RNF8. *Eur J Biochem* **268**, 2725-32.

Ito K, Kato S, Matsuda Y, Kimura M, Okano Y (1999) cDNA cloning, characterization, and chromosome mapping of UBE2E3 (alias UbcH9), encoding an N-terminally extended human ubiquitin-conjugating enzyme. *Cytogenet Cell Genet* **84**, 99-104.



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