

# UBE2E3 (UbcH9) [GST-tagged]

## E2 – Ubiquitin Conjugating Enzyme

Alternate Names: UbcH9, UbcM2, Ubiquitin conjugating enzyme UbcH9

**Cat. No.** 62-0021-020  
**Lot. No.** 1392

**Quantity:** 20 µg  
**Storage:** -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



**CERTIFICATE OF ANALYSIS**

### 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). 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<sup>+</sup> 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  $\alpha$ ,  $\beta$  and  $\gamma$  subunits (Debonneville and Staub, 2004). NEDD4.2 is a negative regulator of ENaC and deletions in the PY motifs of the  $\beta$  and  $\gamma$  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<sup>+</sup> reabsorption by the distal nephron, resulting in hypertension (Abriel *et al.*, 1999).

### References:

Abriel H, Loffing J, Rebhun JF, Pratt JH, Schild L, Horisberger JD, Rotin D, Staub O (1999) Defective regulation of the epithelial Na<sup>+</sup> 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<sup>+</sup> 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.

### 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:** ~50 kDa

**Purity:** >90% by InstantBlue™ SDS-PAGE

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

### Protein Sequence:

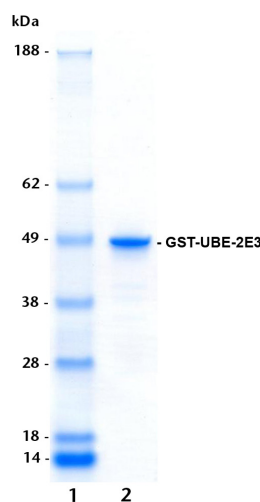
MSPILGYWKIKGLVQPTRLLEYLEEKYEEH  
LYERDEGDKWRNKKFELGLEFPNLPYYIDG  
VKLTQSMARIYIADKHNMLGGCPKER  
AEISMLEGAVLDIRYGVSRAYSKDFETLKVD  
FLSKLPEMLKMFEDRLCHKTYLNGDHTHP  
DFMLYDALDVVLYMDPMCLDAFPKLVCFK  
KRIEAIPIQIDKYLKSSKYIAWPLQGWQATFG  
GGDHPKSDLEVLFFQGPLGSMSSDRQRSD  
DESPSTSSGSSDADQRDPAAPEPEEQEERKP  
SATQQKKNTKLSSKTTAKLSTSAKRIQKE  
LAEITLDPNCSAGPKGDNIYWRSTILGPPGS  
VYEGGVFLDITFSSDYFPKPPKVTFRTRIYHCNIN  
SQGVICLDILKDNWSPALTISKVLLSICSLTDCN  
PADPLVGSATQYLTRAEHDRIARQWTKRYAT

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)  
Protease cleavage site: PreScission™ (LEVLFOVGP)  
UBE2E3 (regular text): Start **bold italics** (amino acid residues 1-207)  
Accession number: NP\_006348.1

### Quality Assurance

#### Purity:

4-12% gradient SDS-PAGE  
InstantBlue™ staining  
lane 1: MW markers  
lane 2: 1 µg GST-UBE2E3



#### Protein Identification:

Confirmed by mass spectrometry.

#### E2-Ubiquitin Thioester Loading Assay:

The activity of GST-UBE2E3 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2E3 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2E3 enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T<sub>0</sub> and T<sub>10</sub> minutes. Sensitivity of the ubiquitin/GST-UBE2E3 thioester bond to the reducing agent DTT was confirmed.



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Lot-specific COA version tracker: v1.0.0