

# UBE2J1<sup>(1-282)</sup> (NCUBE1) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: Ubc6p, CGI-76, NCUBE1, HSPC153, HSPC205

Cat. No. 62-0096-020

Lot. No. 2140

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). UBE2J1 is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Lester *et al.* (2000). UBE2J1 is a 318 amino acid single-pass type IV membrane protein, which can be found localised to the membrane of the endoplasmic reticulum (ER) (Lester *et al.*, 2000). UBE2J1 catalyzes the modification of misfolded membrane proteins with ubiquitin which results in their targeting to the proteasome for degradation (Walter *et al.*, 2001). UBE2J1 has been shown to interact with the E3 ligase ICP0 forming polyubiquitin chains in an *in vitro* polyubiquitylation assay. ICP0 targets substrates such as PML, Sp100, CENP-C, and CENP-A for proteasomal degradation (Everett *et al.*, 1999; Everett *et al.*, 1998; Lomonte *et al.*, 2001; Parkinson and Everett, 2000). Evidence for an association of the UBE2J1 gene locus with Serum creatinine (S CR) levels has been demonstrated. S CR is a biomarker used for the non-invasive assessment of kidney function and it is hoped will provide an insight into the genetic basis of serum creatinine regulatory processes (Pattaro *et al.*, 2010).

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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:** ~35 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** L V P R G S H M A S M T G  
G Q Q M G R G S M E T R Y N L K S P A V K R L M K E A E L  
K D P T D H Y H A Q P L E D N L F E W H F T V R G P P D S D  
F D G G V Y H G R I V L P P E Y P M K P P S I I L L T A N G R  
F E V G K K I C L S I S G H H P E T W Q P S W S I R T A L L A I  
I G F M P T K G E G A I G S L D Y T P E R R A L A K K S Q D  
F C C E G C G S A M K D V L L P L K S G S D S S Q A D Q E A K E  
L A R Q I S F K A E V N S S G K T I S E S D L N H S F S L T  
D L Q D D I P T T F Q G A T A S T S Y G L O N S S A A S F  
H Q P T Q P V A K N T S M S P R Q R R A Q Q S Q R R L S T S P  
D V I Q G H Q P R D N H T

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

Protease cleavage site: Thrombin (LVPR**▼**GS)

UBE2J1 (regular text): Start **bold italics** (amino acid residues 1-282)

Accession number: NP\_057105.2

## Quality Assurance

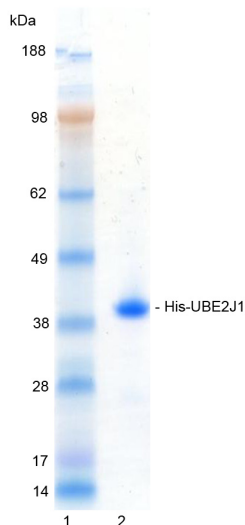
### Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg His-UBE2J1



### Protein Identification:

Confirmed by mass spectrometry.

### E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2J1 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2J1 E2 enzyme via a transthioesteration reaction. Incubation of the UBE1 and His-UBE2J1 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/His-UBE2J1 thioester bond to the reducing agent DTT was confirmed.



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## Background

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### References:

Everett RD, Earnshaw WC, Findlay J, Lomonte P (1999) Specific destruction of kinetochore protein CENP-C and disruption of cell division by herpes simplex virus immediate-early protein Vmw110. *EMBO J* **18**, 1526-38.

Everett RD, Freemont P, Saitoh H, Dasso M, Orr A, Katoria M, Parkinson J (1998) The disruption of ND10 during herpes simplex virus infection correlates with the Vmw110- and proteasome-dependent loss of several PML isoforms. *J Virol* **72**, 6581-91.

Lester D, Farquharson C, Russell G, Houston B (2000) Identification of a family of noncanonical ubiquitin-conjugating enzymes structurally related to yeast UBC6. *Biochem Biophys Res Commun* **269**, 474-80.

Lomonte P, Sullivan KF, Everett RD (2001) Degradation of nucleosome-associated centromeric histone H3-like protein CENP-A induced by herpes simplex virus type 1 protein ICP0. *J Biol Chem* **276**, 5829-35.

Parkinson J, Everett RD (2000) Alpha herpesvirus proteins related to herpes simplex virus type 1 ICP0 affect cellular structures and proteins. *J Virol* **74**, 10006-17.

Pattaro C, De Grandi A, et al. (2010) A meta-analysis of genome-wide data from five European isolates reveals an association of COL22A1, SYT1, and GABRR2 with serum creatinine level. *BMC Med Genet* **11**, 41.

Walter J, Urban J, Volkwein C, Sommer T (2001) Sec61p-independent degradation of the tail-anchored ER membrane protein Ubc6p. *EMBO J* **20**, 3124-31.



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