UBE2J1(1-282) (NCUBE1) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

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

Cat. No. **62-0096-100**
Lot. No. **2140**

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). 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: 100 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

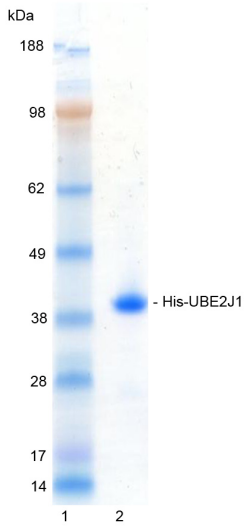
Protein Sequence:

MGSSHHHHHHSSGLVPRGSHMASMTG
GQQMGRGS***M***ETRYNLKSPAVKRLMKEAAEL
KDPTDHYHAQPLEDNLFEWHFTVRGPPDSD
FDGGVYHGRIVLPPEYPMKPPSIILLTANGR
FEVGKKICLSISGHHPETWQPSWSIRTALLAI
IGFMPTKGEGAIGSLDYTPEERRALAKKSQD
FCCEGCGSAMKDVLLPLKSGSDSSQADQEAKE
LARQISFKAEVNSSGKTISESDLNHSFSLT
DLQDDIPTTFQGATASTSYGLQNSSAASF
HQPTQPVAKNTSMSPRQRRAQQQSQRRLSTS
PDVIQGHQPRDNHT

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 transthiolation 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_0 and T_{10} minutes. Sensitivity of the ubiquitin/His-UBE2J1 thioester bond to the reducing agent DTT was confirmed.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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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, Kathoria 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) Alphaherpesvirus 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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