

UBE2J2⁽¹⁻²²⁶⁾ (NCUBE2) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: EC 6.3.2.19, NCUBE2, Non canonical ubiquitin conjugating enzyme 2, PRO2121
UBC6 homolog, Ubc6p

Cat. No. **62-0035-020**
Lot. No. **1375**

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). UBE2J2 is a member of the E2 conjugating enzyme family and the human gene was first described by Lenk *et al.* (2002). UBE2J2 is a 318 amino acid protein that shares 26% identity to its yeast homologue (Lenk *et al.*, 2002). UBE2J2 is localised to the cytoplasmic surface of the Endoplasmic Reticulum (ER) and participates in Endoplasmic Reticulum Associated Degradation (ERAD). It has been demonstrated that expression of a mutant form of UBE2J2 affects ERAD of the T cell receptor and a mutant form of the CFTR protein (Lenk *et al.*, 2002). UBE2J2 has also been identified as the primary cellular E2 recruited by the E3 ligase murine K3 (mK3), and this E2-E3 pair is capable of conjugating ubiquitin on lysine or serine residues of substrates. Interestingly, UBE2J2-mK3 preferentially promotes ubiquitylation of hydroxylated amino acids via ester bonds even when lysine residues are present on wild-type substrates (Wang *et al.*, 2009). Treatment of Sertoli and germ cells of the testies with a proteasome inhibitor results in the colocalisation of the retinoic acid receptor alpha (RARA) to the ER where it interacts with UBE2J2 resulting in its ubiquity-

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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: ~29 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 S S T S S K R A P T T A T Q R L K Q D Y L
R I K K D P V P Y I C A E P L P S N I L E W H Y V V R G
P E M T P Y E G G Y Y H G K L I F P R E F P F K P P S I
Y M I T P N G R F K C N T R L C L S I T D F H P D T W N
P A W S V S T I L T G L L S F M V E K G P T L G S I E T S
D F T K R Q L A V Q S L A F N L K D K V F C E L F P E V V
E E I K Q K Q A Q D E L S S R P Q T L P L P D V V P D G E T H
L V Q N G I Q L L N G H A P G A V P N L A G L Q Q A N R H H

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

Protease cleavage site: Thrombin (L**VPR**↓**GS**)

UBE2J2 (regular text): Start **bold italics** (amino acid residues 1-226)

Accession number: NP_477515.2

Quality Assurance

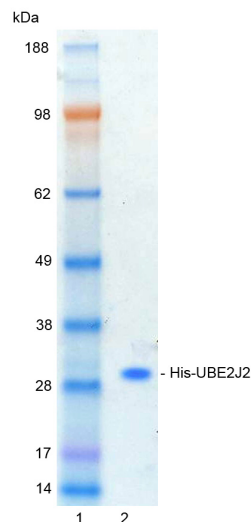
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg His-UBE2J2



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2J2 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2J2 E2 enzyme via a transthioesteration reaction. Incubation of the UBE1 and His-UBE2J2 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-UBE2J2 thioester bond to the reducing agent DTT was confirmed.



www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT

International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

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

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Lot-specific COA version tracker: v1.0.0

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Background

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lation and degradation via the ERAD pathway (Zhu *et al.*, 2010).

References:

Lenk U, Yu H, Walter J, Gelman MS, Hartmann E, Kopito RR, Sommer T (2002) A role for mammalian Ubc6 homologues in ER-associated protein degradation. *J Cell Sci* **115**, 3007-14.

Wang X, Herr RA, Rabelink M, Hoeben RC, Wiertz EJ, Hansen TH (2009) Ube2j2 ubiquitinates hydroxylated amino acids on ER-associated degradation substrates. *J Cell Biol* **187**, 655-68.

Zhu L, Santos NC, Kim KH (2010) Disulfide isomerase glucose-regulated protein 58 is required for the nuclear localization and degradation of retinoic acid receptor alpha. *Reproduction* **139**(4), 717-31.



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Dundee, Scotland, UK

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International: +44 (0) 1382 381147 (9AM-5PM UTC)
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Email: tech.support@ubiquigent.com

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