# 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

FOR RESEARCH USE ONLY

Quantity: 20 µg Storage: -70°C

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 ubiguitin on lysine or serine residues of substrates. Interestingly, UBE2J2-mK3 preferentially promotes ubiguitylation 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

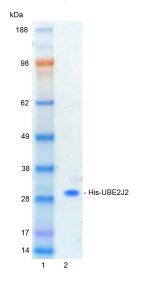
Purity: >98% by InstantBlue™ SDS-PAGE

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

## **Quality Assurance**

#### Purity:

4-12% gradient SDS-PAGE InstantBlue™ staining Lane 1: MW markers Lane 2: 1 μg His-UBE2J2



#### **Protein Sequence:**

MGSSHHHHHHSSGLVPRGSHMASMTG GQQMGRGSMSSTSSKRAPTTATQRLKQDYL RIKKDPVPYICAEPLPSNILEWHYVVRG PEMTPYEGGYYHGKLIFPREFPFKPPSI YMITPNGRFKCNTRLCLSITDFHPDTWN PAWSVSTILTGLLSFMVEKGPTLGSIETS DFTKRQLAVQSLAFNLKDKVFCELFPEVV EEIKQKQKAQDELSSRPQTLPLPDVVPDGETH LVQNGIQLLNGHAPGAVPNLAGLQQANRHH

Tag (**bold text**): N-terminal His Protease cleavage site: Thrombin (<u>LVPR▼GS</u>) UBE2J2 (regular text): Start *bold italics* (amino acid residues 1-226) Accession number: NP\_477515.2

### 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 transthiolation 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,  $T_0$  and  $T_{10}$  minutes. Sensitivity of the ubiquitin/His-UBE2J2 thioester bond to the reducing agent DTT was confirmed.



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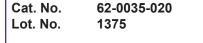
Lot-specific COA version tracker: v1.0.0

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## **CERTIFICATE OF ANALYSIS Page 2 of 2**

## Background

Continued from page 1

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 glucoseregulated protein 58 is required for the nuclear localization and degradation of retinoic acid receptor alpha. *Reproduction* **139**(4), 717-31.



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