

UBE2J2⁽¹⁻²²⁶⁾ (NCUBE2) [GST-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. 62-0036-020

Lot. No. 1397

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: ~52 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

MSPILGYWKIKGLVQPTRLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSR IAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHSVTHPD
FMLYDALDVLVLYMDPMLDAFPKLVCFK
KRIEAIPOIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLVFGPPLGMSSTSSKRAPT
TATQRLKQDYLRICKDPVPICAEPLPSNILE
WHYVVRGPEMTPYEGGYHKGKLIFFREFP
FKPSSIYMITPNGRFKCNTRLCLSLTDFHP
DTWNPASVSTILTGLLSFMVEKGP TLGSI
ETSDFTKRQLAVQSLAFNLKDKVFCELFPEVV
EEIKQKQKAQDELSSRPQTLPLPDVVPDGETH
LVQNGIQLLNHPGAVPNLAGLQQANRHH

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

Protease cleavage site: PreScission™ (LEVLVFGP)

UBE2J2 (regular text): Start **bold italics** (amino acid residues 1-226) Accession number: NP_477515.2

Quality Assurance

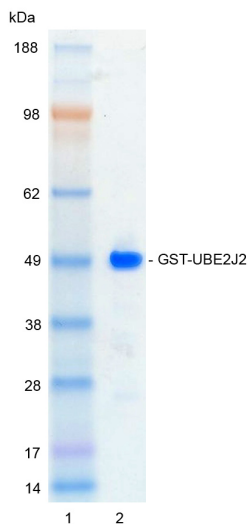
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg GST-UBE2J2



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of GST-UBE2J2 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2J2 E2 enzyme via a transthioylation reaction. Incubation of the UBE1 and GST-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/GST-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

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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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