# UBE2N (UBC13) [untagged] E2 – Ubiquitin Conjugating Enzyme

Alternate Names: Bendless homolog of, Bendless-like ubiquitin conjugating enzyme, MGC131857, MGC8489, UBC13, UbcHBEN

Cat. No.	62-0047-020
Lot. No.	30117

The enzymes of the ubiquitylation

pathway play a pivotal role in a num-

ber of cellular processes including

regulated and targeted proteasomal

degradation of substrate proteins.

Three classes of enzymes are in-

volved in the process of ubiquityla-

tion; activating enzymes (E1s), con-

jugating enzymes (E2s) and protein

ligases (E3s). UBE2N is a member of

the E2 conjugating enzyme family and cloning of the human gene was first

described by Yamaguchi et al. (1996). The human UBE2N sequence shares

80% identity with the Drosophila 'bendless' gene product. In yeast, UBE2N

forms a specific heteromeric complex

with MMS2 a signalling component of the RAD6 pathway. The RAD6 path-

way is central to DNA repair and two

major components of this pathway

are RAD6 and the MMS2/UBE2N

heterodimer which are recruited to

chromatin by the RING finger proteins

RAD18 and RAD5, respectively (Hof-

mann and Pickart, 1999). Proliferating

Cell Nuclear Antigen (PCNA) is modi-

fied by lys-63-linked polyubiquityla-

tion, which requires MMS2, UBE2N

and RAD5. Depletion of UBE2N in

vitro results in severe growth defects

caused by chromosome instability, as

well as hypersensitivity to UV and ion-

izing radiation, this is consistent with a conserved role for UBE2N in RAD6/

repair (Zhao et al., 2007). Cytokine

post-replication

Quantity: 20 µg Storage: -70°C

NOT FOR USE IN HUMANS

FOR RESEARCH USE ONLY

Background

# Physical Characteristics

Species: human

Source: Sf21 insect cell-baculovirus 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: ~17 kDa

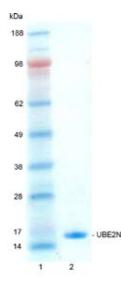
Purity: >95% by InstantBlue™ SDS-PAGE

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

# **Quality Assurance**

### **Purity:**

4-12% gradient SDS-PAGE InstantBlue<sup>™</sup> staining Lane 1: MW markers Lane 2: 1 µg UBE2N



## **Protein Sequence:**

**UBIQUIGENT**<sup>™</sup>

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GAMGS**A**GLPRRIIKETQRLLAEPVPGIKAEP DESNARYFHVVIAGPQDSPFEGGTFKLEL FLPEEYPMAAPKVRFMTKIYHPNVDKLGRI CLDILKDKWSPALQIRTVLLSIQALLSAPNPD DPLANDVAEQWKTNEAQAIETARAWTRLYAMN ΝI

The residues underlined remain after cleavage and removal of the purification tag. UBE2N (regular text): Start bold italics (amino acid residues 2-152) Accession number: NP\_003339

## **Protein Identification:**

Confirmed by mass spectrometry.

### E2-Ubiquitin Thioester Loading Assay:

The activity of UBE2N was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the UBE2N E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and UBE2N 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/UBE2N thioester bond to the reducing agent DTT was confirmed.



RAD18-dependent

Continued on page 2

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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

receptor signalling results in complex formation of protein kinases such as CD40 with TRAF2 and TRAF3, UBE2N, cellular inhibitor of apoptosis protein-1 (CIAP1) and -2 (CIAP2), IKK-α and MEKK1. Translocation of a TRAF2, UBE2N, and IKK-a complex from the membrane to the cytosol is initiated by a CIAP1/CIAP2-induced degradation of TRAF3 which results in activation of MEKK1 and MAP kinase cascades (Matsuzawa et al., 2008). Heterozygous UBE2N mice exhibit selectively impaired activation of signal transduction pathways initiated by TNFr and show reduced ubiguitylation of TRAF6. Reducing UBE2N activity may have therapeutic uses in controlling inflammatory responses. (Matsuzawa et al., 2008)

# **References:**

Hofmann RM, Pickart CM (1999) Noncanonical MMS2-encoded ubiquitinconjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. *Cell* **96**, 645-53.

Matsuzawa A, Tseng PH, Vallabhapurapu S, Luo JL, Zhang W, Wang H, Vignali DA, Gallagher E, Karin M (2008) Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science* **321**, 663-8.

Yamaguchi T, Kim NS, Sekine S, Seino H, Osaka F, Yamao F, Kato S (1996) Cloning and expression of cDNA encoding a human ubiquitin-conjugating enzyme similar to the Drosophila bendless gene product. *J Biochem* **120**, 494-97.

Zhao GY, Sonoda E, Barber LJ, Oka H, Murakawa Y, Yamada K, Ikura T, Wang X, Kobayashi M, Yamamoto K, Boulton SJ, Takeda S (2007) A critical role for the ubiquitin-conjugating enzyme Ubc13 in initiating homologous recombination. *Mol Cell* **25**, 663-75.



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