UBE2N (UBC13) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

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

Cat. No. **62-0045-020**
Lot. No. **1377**

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 proteosomal 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). 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 pathway 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 (Hofmann and Pickart, 1999). Proliferating Cell Nuclear Antigen (PCNA) is modified by Lys-63-linked polyubiquitylation, 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 ionizing radiation, this is consistent with a conserved role for UBE2N in RAD6/RAD18-dependent post-replication repair (Zhao *et al.*, 2007). Cytokine 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-α 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

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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: ~20 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

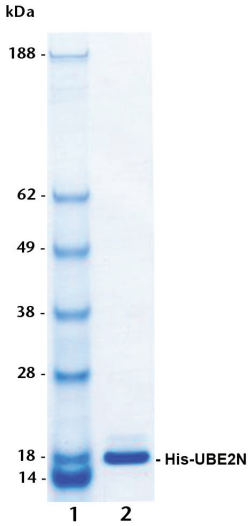
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSYYHHHHHHDYDIPTTENLYFQGAMG
S***A***GLPRRIIKETQRLLAEPVPGIKAEPDESNARY
FHVVIAGPQDSPFEGGTFKLELFLPEEYPMAAP
KVRFMTKIYHPNVDKLGRICLDILKDKWSPALQ
IRTVLLSIQALLSAPNPDDPLANDVAEQWKT
NEAQAIETARAWTRLYAMNNI

Tag (**bold text**): N-terminal His
Protease cleavage site: TEV (ENLYFQ▼G)
UBE2N (regular text): Start ***bold italics*** (amino acid residues 2-152)
Accession number: NP_003339

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg His-UBE2N



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of His-UBE2N was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2N E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-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/His-UBE2N 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: v1.0.1

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Background

Continued from page 1

mice exhibit selectively impaired activation of signal transduction pathways initiated by TNFr and show reduced ubiquitylation 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 ubiquitin-conjugating 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, et al. (2007) A critical role for the ubiquitin-conjugating enzyme Ubc13 in initiating homologous recombination. *Mol Cell* **25**, 663-75.

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Lot-specific COA version tracker: v1.0.1