UBE2N (UBC13) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

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

Cat. No. **62-0046-100**
Lot. No. **1401**

Quantity: 100 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). 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

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Physical Characteristics

Species: human

Source: *E. coli* expression

Quantity: 100 µg

Concentration: 1 mg/ml

Formulation: 50 mM HEPES pH 7.5, 150 mM sodium chloride, 2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~44 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

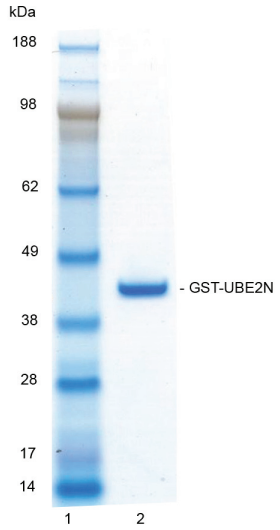
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKSDLEVLFQGPLGS**A**GLPRRI
IKETQRLLAEPVPGIKAEPDESNARYFHVVI
AGPQDSPFEGGTFKLELFLPEEYPMAAPKVR
FMTKIYHPNVDKLGRICLDILKDKWSPALQ
IRTVLLSIQALLSAPNPDDPLANDVAEQWKT
NEAQAIETARAWTRLYAMNNI

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2N (regular text): Start ***bold italics*** (amino acid residues 2-152)
Accession number: AAH03365

Quality Assurance

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



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2N was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2N E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2N enzymes in the presence of ubiquitin and ATP at 30°C was compared at two time points, T_0 and T_{10} minutes. The sensitivity of this ubiquitin/GST-UBE2N thioester bond to the reducing agent DTT was demonstrated.

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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

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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 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, 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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