UBE2K (UbcH1) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: Huntingtin-Interacting Protein 2; HIP2; E2-25K

Cat. No. **62-0038-020**
Lot. No. **1398**

Quantity: 20 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

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). UBE2K is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Kalchman *et al.* (1996). Human UBE2K shares 100% amino acid identity with bovine UBE2K and significant homology with yeast UBE2K, UBE2D2 and UBE2D1 (Kalchman *et al.*, 1996). Interaction and selective binding of UBE2K to the N-terminus of huntingtin, the causal gene product in Huntington's disease has been demonstrated (Kalchman *et al.*, 1996). In a yeast 2 hybrid screen binding of the RING finger protein RNF2 to UBE2K has also been determined (Christensen *et al.*, 2007; Lee *et al.*, 2001). UBE2K binds directly to the BRCA1 RING motif and is active with BRCA1-BARD1 in *in vitro* autoubiquitylation assays (Christensen *et al.*, 2007). UBE2K directs the synthesis of Lys63- or Lys48-linked ubiquitin chains on BRCA1. UBE2K has been found to synthesize *in vitro* unanchored Lys(48)-linked poly-ubiquitin chains from mono- or poly-ubiquitin, E1, and ATP; thus, UBE2K has distinct binding sites for donor and acceptor (poly) Ub (Yao and Cohen, 2000). UBE2K was identified in a screen for novel SUMO targets, however attachment of SUMO to UBE2K *in vitro* severely impairs ubiquitin thioester and unanchored ubiquitin chain formation (Pichler *et al.*, 2005). The ubiquitin-proteasome system malfunction in Alzheimer's disease (AD) has been attributed to neurotoxicity and proteasome inhibition by Abeta, which is mediated by an increase in the levels of UBE2K found

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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: ~49 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

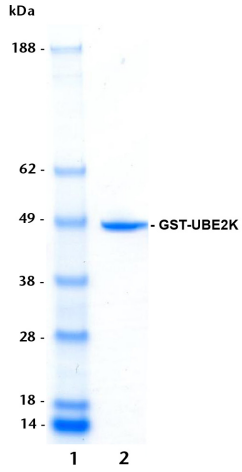
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY EEHLYERDEGDKWRNKKFELGLEFPNLPYY IDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKD FETLKVDFLSKLPEMLKMFEDRLCHK TYLNGDHVTHPDFMLYDALDVVLYMDP MCLDAFPKLVCFKKRIEAIPQIDKYLKSSKY IAWPLQGWQATFGGGDHPPKSDLEV LFQGPLG***M***ANIAVQRIKREFKEVLKSEETSKN QIKVDLVDENFTELRGEIAGPPDTPYEGGRY QLEIKIPETYPFNPPKVRFITKIWHPNISSVT GAICLDILKDQWAAAMTLRTVLLSLQAL LAAAEPDDPQDAVVANQYKQNPEMFKQTAR LWAHVYAGAPVSSPEYTKKIENLCAMGFDR NAVIVALSSKSWDVETATELLLSN**

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2K (regular text): Start ***bold italics*** (amino acid residues 1-200)
Accession number: NP_005330

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg GST-UBE2K



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2K was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2K E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2K enzymes in the presence of ubiquitin and ATP at 30˚C was compared at three time points, T_0, T_3 and T_6 hours. Sensitivity of the ubiquitin/GST-UBE2K 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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in the brains of patients with AD. UBE2K's contribution to neurotoxicity is mediated by a ubiquitin B mutant (UBB+1), a potent inhibitor of proteasomes found in patients with AD (Song and Jung, 2004).

References:

Christensen DE, Brzovic PS, Klevit RE (2007) E2-BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages. *Nat Struct Mol Biol* **14**, 941-8.

Kalchman MA, Graham RK, Xia G, Koide B, Hodgson JG, Graham KC, Goldberg YP, Gietz RD, Pickart CM, Haydan MR (1996) Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. *J Biol Chem* **271**, 19385-94.

Lee SJ, Choi JY, Sung YM, Park H, Rhim H, Kang S (2001) E3 ligase activity of RING finger proteins that interact with Hip-2, a human ubiquitin-conjugating enzyme. *FEBS Lett* **503**, 61-4.

Pichler A, Knipscheer P, Oberhofer E, van Dijk WJ, Korner R, Olsen JV, Jentsch S, Melchior F, Sixma TK (2005) SUMO modification of the ubiquitin-conjugating enzyme E2-25K. *Nat Struct Mol Biol* **12**, 264-9.

Song S, Jung YK (2004) Alzheimer's disease meets the ubiquitin-proteasome system. *Trends Mol Med* **10**, 565-70.

Yao T, Cohen RE (2000) Cyclization of polyubiquitin by the E2-25K ubiquitin conjugating enzyme. *J Biol Chem* **275**, 36862-8.

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