

UBE2K (Ubc1) [6His-tagged]

E2 – Ubiquitin Conjugating Enzyme

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

Cat. No. 62-0079-020

Lot. No. 1830

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). 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* auto-ubiquitylation 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).

Continued on page 2

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: ~25 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

Protein Sequence:

MGSSHHHHHSSGLEVLFGPGSMANIA
VQRIKREFKEVLKSEETSKNQIKVDLVDEN
FTELGRGEIAGPPDTPYEGGRYQLEIKIP
ETYPFPNPKVRFITKIWHPNISSVT
GAICLDILKDQWAAAMTLRTVLLSLQAL
LAAAEPDDPQDAVVANQYKQNPENFKQTAR
LWAHVYAGAPVSSPEYTKKIENLCAMGFDR
NAVIVALSSKSWDDETATELLLSN

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

Protease cleavage site: PreScission™ (LEVLFG▼GP)

UBE2K (regular text): Start **bold italics** (amino acid residues 1-200)

Accession number: NP_005330

Quality Assurance

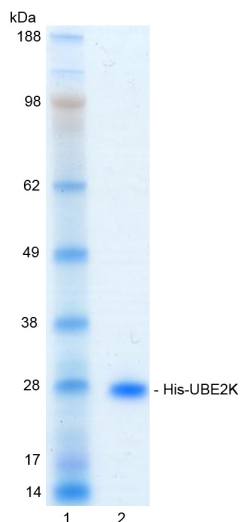
Purity:

4-12% gradient SDS-PAGE

InstantBlue™ staining

Lane 1: MW markers

Lane 2: 1 µg His-UBE2K



Protein Identification:

Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:

The activity of His-UBE2K was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the His-UBE2K E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and His-UBE2K 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/His-UBE2K thioester bond to the reducing agent DTT was confirmed.



www.ubiquigent.com
Dundee, Scotland, UK

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International: +1-617-245-0003
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT

International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

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Lot-specific COA version tracker: v1.0.0

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Continued from page 1

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 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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