UBE2E2 (UbcH8) [GST-tagged]

E2 - Ubiquitin or ISG15 Conjugating Enzyme

Alternate Names: UbcH8

Cat. No. **62-0087-020**
Lot. No. **1839**

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 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). UBE2E2 is a member of the E2 ubiquitin-conjugating enzyme family and cloning of the human gene was first described by Kimura *et al.* (1997). The Ubc domain of UBE2E2 shares over 90% identity with human UBE2E1, mouse UbcM2, and *Drosophila* UbcD2 (Kimura *et al.*, 1997). UBE2E2 has been shown to ubiquitylate the E3 ligase E6AP by binding to its HECT domain (Kumar *et al.*, 1997). A yeast two hybrid screen identified two UBE2E2 binding proteins, UbcH7-Associated Protein (H7-AP1) and Human Homologue of *Drosophila* ARIadne (HHARI); both of these proteins are characterized by the presence of a RING finger and In Between RING finger (IBR) domains (Moynihan *et al.*, 1999). Studies using deletion mutants of UBE2E2 and two point mutants - ARA54 and C220S - and RNF8 C403S, have demonstrated that ARA54 and RNF8 ring finger proteins interact with the Ubc domain of UBE2E2 (Ito *et al.,* 2001). UBE2E2 binds directly to the BRCA1 RING motif of the human heterodimeric RING E3 ligase complex BRCA1-BARD1 and is active in causing autoubiquityla-

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: ~49 kDa

Purity: >90% by InstantBlue™ SDS-PAGE

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

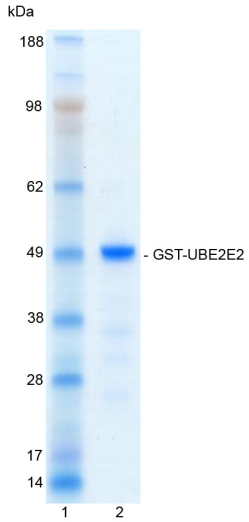
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEM
LKMFEDRLCHKTYLNGDHVTHPDFMLYDALDV
VLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEV
LFQGPLGS***M***STEAQRVDDSPSTSGGSSDGDQRES
VQQEPEREQVQPKKKEGKISSKTAAKLST
SAKRIQKELAEITLDPPPNCSAGPKGDNIYE
WRSTILGPPGSVYEGGVFFLDITFSPDYPFKP
PKVTFRTRIYHCNINSQGVICLDILKDNWSPAL
TISKVLLSICSLLTDCNPADPLVGSIATQYMT
NRAEHDRMARQWTKRYAT

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2E2 (regular text): Start ***bold italics*** (amino acid residues 1-201)
Accession number: NP_689866.1

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-UBE2E2



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2E2 was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2E2 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2E2 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/GST-UBE2E2 thioester bond to the reducing agent DTT was confirmed.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
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

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

UBE2E2 (UbcH8) [GST-tagged]

E2 - Ubiquitin or ISG15 Conjugating Enzyme

Alternate Names: UbcH8

Cat. No.	**62-0087-020**	Quantity:	20 µg
Lot. No.	**1839**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

Continued from page 1

tion *in vitro* (Christensen *et al.*, 2007). UBE2E2 has also been shown to bind the ubiquitin-protein ligase Parkin via its C-terminal ring-finger domain, resulting in ubiquitylation of the synaptic vesicle associated protein CDCrel-1 (Zhang *et al.*, 2000).

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.

Ito K, Adachi S, Iwakami R, Yasuda H, Muto Y, Seki N, Okano Y (2001) N-Terminally extended human ubiquitin-conjugating enzymes (E2s) mediate the ubiquitination of RING-finger proteins, ARA54 and RNF8. *Eur J Biochem* **268**, 2725-32.

Kimura M, Hattori T, Matsuda Y, Yoshioka T, Sumi N, Umeda Y, Nakashima S, Okano Y (1997) cDNA cloning, characterization, and chromosome mapping of UBE2E2 encoding a human ubiquitin-conjugating E2 enzyme. *Cytogenet Cell Genet* **78**, 107-11.

Kumar S, Kao WH, Howley PM (1997) Physical interaction between specific E2 and Hect E3 enzymes determines functional cooperativity. *J Biol Chem* **272**, 13548-54.

Moynihan TP, Ardley HC, Nuber U, Rose SA, Jones PF, Markham AF, Scheffner M, Robinson PA (1999) The ubiquitin-conjugating enzymes UbcH7 and UbcH8 interact with RING finger/IBR motif-containing domains of HHARI and H7-AP1. *J Biol Chem* **274**, 30963-8.

Zhang Y, Gao J, Chung KK, Huang H, Dawson VL, Dawson TM (2000) Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc Natl Acad Sci U S A* **97**, 13354-9.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
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

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0