UBE2W (Ubc16) [GST-tagged]

E2 – Ubiquitin Conjugating Enzyme

Alternate Names: FLJ11011

Cat. No. **62-0091-100**
Lot. No. **1843**

Quantity: 100 µg
Storage: -70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 1

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the 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). UBE2W is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Yin *et al.* (2006). UBE2W comprises 7 exons and there are two Nuclear Localisation Signals (NLS) located in the c-terminus of the UBC domain (Yin *et al.*, 2006). Interaction of UBE2W with the human heteromeric RING E3 BRCA1-BARD1 has been demonstrated using yeast two-hybrid screening. UBE2W binds directly to the RING motif of BRCA-1 causing autoubiquitylation of BRCA1-BARD1 and monoubiquitylation of BRCA1 alone *in vitro* (Christensen *et al.*, 2007). UBE2W also interacts with UBE1 and the E3 ligase FANCL to monoubiquitylate FANCD2 *in vitro* (Alpi *et al.*, 2008).

References:

Alpi AF, Pace PE, Babu MM, Patel KJ (2008) Mechanistic insight into site-restricted monoubiquitination of FANCD2 by Ube2t, FANCL, and FANCI. *Mol Cell* **32**, 767-77.

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.

Yin G, Ji C, Wu T, Shen Z, Xu X, Xie Y, Mao Y (2006) Cloning, characterization and subcellular localization of a gene encoding a human Ubiquitin-conjugating enzyme (E2) homologous to the Arabidopsis thaliana UBC-16 gene product. *Front Biosci* **11**, 1500-7.

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: ~45 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

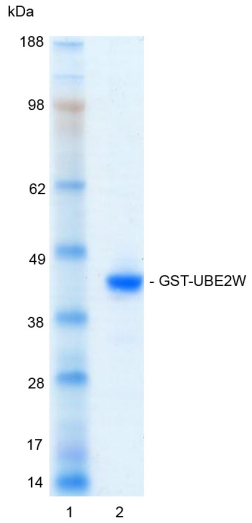
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEM
LKMFEDRLCHKTYLNGDHVTHPDFMLYDALDV
VLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEV
LFQGPLGS***M***ASMQTTGRRVEVWFPKRLQKELLA
LQNDPPPGMTLNEKSVQNSITQWIVDMEGAPGT
LYEGEKFQLLFKFSSRYPFDSPQVMFTGENIPVH
PHVYSNGHICLSILTEDWSPALSVQSVCLSI
ISMLSSCKEKRRPPDNSFYVRTCNKNPKKTKWW
YHDDTC

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2W (regular text): Start ***bold italics*** (amino acid residues 1-162)
Accession number: NP_001001481.1

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-UBE2W



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2W was validated by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2W E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2W 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-UBE2W thioester bond to the reducing agent DTT was confirmed.

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Lot-specific COA version tracker: v1.0.0