UBE2L6 (UbcH8) [GST-tagged]

E2 - Ubiquitin or ISG15 Conjugating Enzyme

Alternate Names: MGC40331, Retinoic acid induced gene B protein, RIG-B, UbcH8, Ubiquitin conjugating enzyme UbcH8

Cat. No. **62-0043-020**
Lot. No. **1400**

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 ubiquitination; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). UBE2L6 is a member of the E2 conjugating enzyme family, identification and cloning of the gene from a yeast 2 hybrid screen was first described by Kumar *et al.* (1997). Human UBE2L6 has been mapped to chromosome 11q12 and shares 46% homology with UBE2L3 (Recombinant UBE2L6 forms thiol ester complexes with ubiquitin *in vitro* and is able to transfer ubiquitin to E6AP (Ardley *et al.*, 2000). UBE2L6 has also been shown to conjugate the ubiquitin like protein (Ubl) ISG15. *In vitro* assays and RNA interference have shown that of the ubiquitin E2 enzymes tested UBE2L6 is a major E2 enzyme for ISG15 conjugation (Li *et al.*, 2006; Zhao *et al.*, 2004). The nuclear matrix protein Msx2-interacting nuclear target protein (MINT) regulates the expression of key transcriptional effectors in diverse signalling pathways and has been identified as a binding partner of UBE2L6 by co-immunoprecipitation and GST pull down (Li *et al.*, 2006). Degradation of the chromosomal translocation products AML1-ETO and PML-RAR alpha which contribute to the pathogenesis of leukaemias is mediated by the E2 en-

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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: ~44.6 kDa

Purity: >98% by InstantBlue™ SDS-PAGE

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

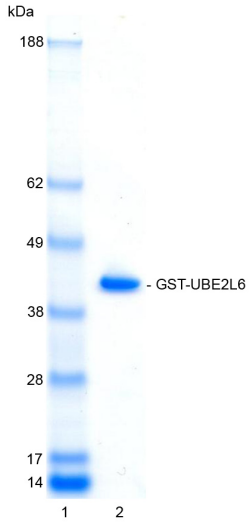
Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEM
LKMFEDRLCHKTYLNGDHVTHPDFMLYDALDV
VLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEV
LFQGPLGS***M*MASMRVVKELEDLQKKPPPYLRN
LSSDDANVLVWHALLLPDQPPYHLKAFNLRISF
PPEYPFKPPMIKFTTKIYHPNVDENGQICLPIIS
SENWKPCTKTCQVLEALNVLVNRPNIREPLRMD
LADLLTQNPELFRKNAEEFTLRFGVDRPS

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2L6 (regular text): Start ***bold italics*** (amino acid residues 1-153)
Accession number: NP_004214

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-UBE2L6



Protein Identification:
Confirmed by mass spectrometry.

E2-Ubiquitin Thioester Loading Assay:
The activity of GST-UBE2L6 was tested by loading E1 UBE1 activated ubiquitin onto the active cysteine of the GST-UBE2L6 E2 enzyme via a transthiolation reaction. Incubation of the UBE1 and GST-UBE2L6 enzymes in the presence of ubiquitin and ATP at 30˚C was compared at two time points, T_0 and T_{10} minutes. Under these conditions tested no GST-UBE2L6 ubiquitin thioester loading was observed.

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

Lot-specific COA version tracker: v1.0.0

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Background

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zyme UBE2L6 and the E3 ligase SIAH-1. Thus, UBE2L6 could be a potential therapeutic target in the treatment of leukaemia (Kramer *et al.*, 2008).

References:

Ardley HC, Rose SA, Tan N, Leek JP, Markham AF, Robinson PA (2000) Genomic organization of the human ubiquitin-conjugating enzyme gene, UBE2L6 on chromosome 11q12. *Cytogenet Cell Genet* **89**, 137-40.

Kramer OH, Muller S, Buchwald M, Reichardt S, Heinzel T (2008) Mechanism for ubiquitylation of the leukemia fusion proteins AML1-ETO and PML-RARalpha. *FASEB J* **22**, 1369-79.

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.

Li J, Wang J, Yang X, Qin H, Dong X, Zhu Y, Liang L, Liang Y, Han H (2006) The Spen homolog Msx2-interacting nuclear target protein interacts with the E2 ubiquitin-conjugating enzyme UbcH8. *Mol Cell Biochem* **288**, 151-7.

Zhao C, Beaudenon SL, Kelley ML, Waddell MB, Yuan W, Schulman BA, Huibregtse JM, Krug RM (2004) The UbcH8 ubiquitin E2 enzyme is also the E2 enzyme for ISG15, an IFN-alpha/beta-induced ubiquitin-like protein. *Proc Natl Acad Sci U S A* **101**, 7578-82.

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Lot-specific COA version tracker: v1.0.0