UBE2V1 (Uev1) [GST-tagged]

E2 - Ubiquitin Conjugating Enzyme

Alternate Names: CIR1, CROC1, TRAF6-regulated IKK activator 1 beta Uev1A, UBE2V, Ubiquitin-conjugating enzyme variant Kua, Uev1, UeV1A

Cat. No. **62-0102-020**
Lot. No. **30067**

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). UBE2V1 is a member of the E2 conjugating enzyme family and cloning of the human gene was first described by Rothofsky and Lin (1997). UBE2V1 is also known as Uev1A and there is an additional isoform of Uev1A referred to as Uev1B. UBE2V1 shares 90% sequence identity with UBE2V2 in its C-terminal domain but lacks the active cysteine site critical for E2 catalytic activity. Constitutive expression of exogenous Uev1A (UBE2V1) protein in colon carcinoma cells inhibits differentiation and induces a change in cell cycle behaviour which is associated with an inhibition of the mitotic kinase CDK1 (Sancho *et al.*, 1998). UBE2V1 forms a stable high affinity complex alongside the E2 conjugating enzyme Ube2N for the assembly of Lys-63-linked ubiquitin chains and it is by this mechanism the complex known as TRIKA1 mediates IKK activation together with TRAF6. The TRIKA2 complex comprising TAK1, TAB1 and TAB2 activates IKK in a TRIKA1 dependent manner. TAK1 phosphorylation of MKK6 has been shown to activate the JNK-p38 kinase pathway and is regulated by the Lys63-linked polyubiquitin chains on TRIKA1 (Wang *et al.*, 2001). Distinct functions amongst the UBE2V1 variants exist, the yeast homologue Mms2 with Ube2N is involved in DNA damage repair whereas human UBE2V1 activates the NFκB pathway. These novel mechanisms are likely due to the alternative Lys-63-linked polyubiquitylation modulated by Ube2N (Andersen *et*

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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: ~46 kDa

Purity: >97% by InstantBlue™ SDS-PAGE

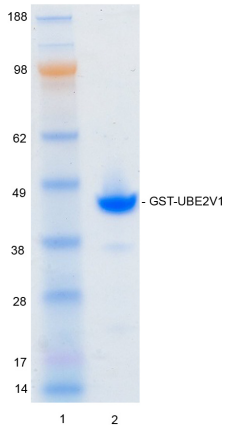
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence:
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYY
IDGDVKLTQSMAIIRYIADKHNMLGGCPKER
AEISMLEGAVLDIRYGVSRIAYSKDFETLKVD
FLSKLPEMLKMFEDRLCHKTYLNGDHVTHP
DFMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKSDLEVLFQGPLGS***P***GEVQA
SYLKSQSKLSDEGRLEPRKFHCKGVKVPRN
FRLLEELEEGQKGVGDGTVSWGLEDDED
MTLTRWTGMIIGPPRTIYENRIYSLKIECGPKY
PEAPPFVRFVTKINMNGVNSSNGVVDPRAIS
VLAKWQNSYSIKVVLQELRRLMMSKENM
KLPQPPEGQCYSN

Tag (**bold text**): N-terminal glutathione-S-transferase (GST)
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
UBE2V1 (regular text): Start ***bold italics*** (amino acid residues 2-170)
Accession number: AAG24229

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
lane 1: MW markers
lane 2: 1 µg GST-UBE2V1



Protein Identification:
Confirmed by mass spectrometry.

E3 Autoubiquitylation Ligase Assay:
The activity of GST-UBE2V1 was validated in an E3 autoubiquitylation assay. Incubation of UBE1, UBE2N and CHIP - with and without GST-UBE2V1 - in the presence of ubiquitin and ATP at 30˚C was compared at two time points, T_0 and T_{60} minutes. Polyubiquitin chains were detected by Western blot using a polyclonal anti-ubiquitin antibody. The ability of GST-UBE2V1 to promote the formation of polyubiquitin chains 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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al., 2005). UBE2V1 has also been shown to upregulate Bcl2 providing evidence that UBE2V1 is a potential protooncogene (Syed *et al.*, 2006). In addition, significant upregulation of UBE2V1 expression has been shown in macroarray analyses of 128 genes derived from nephrectomy samples of kidney transplant recipients with chronic allograft nephropathy and acute rejection (Nogueira *et al.*, 2009).

References:

Andersen PL, Zhou H, Pastushok L, Moraes T, McKenna S, Ziola B, Ellison MJ, Dixit VM, Xiao W (2005) Distinct regulation of Ubc13 functions by the two ubiquitin-conjugating enzyme variants Mms2 and Uev1A. *J Cell Biol* **170**, 745-55.

Nogueira E, Ponciano VC, Naka EL, Marques GD, Cenedeze MA, Camara NO, Pacheco-Silva A (2009) Toll-like receptors-related genes in kidney transplant patients with chronic allograft nephropathy and acute rejection. *Int Immunopharmacol* **9**, 673-6.

Rothofsky ML, Lin SL (1997) CROC-1 encodes a protein which mediates transcriptional activation of the human FOS promoter. *Gene* **195**, 141-9.

Sancho E, Vila MR, Sánchez-Pulido L, Lozano JJ, Paciucci R, Nadal M, Fox M, Harvey C, Bercovich B, Loukili N, Ciechanover A, Lin SL, Sanz F, Estivill X, Valencia A, Thomson TM (1998) Role of UEV-1, an inactive variant of the E2 ubiquitin-conjugating enzymes, in *in vitro* differentiation and cell cycle behavior of HT-29-M6 intestinal mucosecretory cells. *Mol Cell Biol* **18**, 576-89.

Syed NA, Andersen PL, Warrington RC, Xiao W (2006) Uev1A, a ubiquitin conjugating enzyme variant, inhibits stress-induced apoptosis through NF-kappaB activation. *Apoptosis* **11**, 2147-57.

Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ (2001) TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* **412**, 346-51.

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