

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of GST-UBE2V1**

**Enzyme description:-** UBE2V1 (2-170) full length without Start Met

**Clone number:-** DU1813

**Source:-** BL21 recombinant

**Tag:-** N-terminal GST-tag

**Purification method:-** GSH-Sepharose

**Expression level:-** 3mg/L

**Calculated molecular mass:-**

Monoisotopic 45970 Da

Average Mass 45999 Da

[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-** 6.79

**Purity:-** 90%

**Enzyme storage buffer:-**

50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT

**Storage temperature:-** -80°C

**Assay:-**

Production of free K63 linked Ub-chains with Ubiquitin, UBE1 and UBE2N in the presence of Mg-ATP

Assay buffer: 50mM HEPES, pH 7.5, 1mM DTT

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**Clone Data Sheet**

**GST-UBE2V1**

**Protein** UBE2V1 2-170  
**Synonyms** Uev1A, CIR1, CROC1  
**Clone Number** DU1813  
**Species** Human  
**Accession Number** Protein: NP\_068823      DNA: NM\_021988  
**Tags** N-terminal GST-tag

Aminoacid sequence of the expressed protein  
MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELGL  
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGAVL  
DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH  
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA  
WPLQGWQATFGGGDHPKSDLEVLFOGPLGSPGEVQASYLKSQSKLSDEG  
**RLEPRKFHCKGVKVPNRFLLEELEEGQKGVGDGTVSWGLEDDEDMTLTR**  
**WTGMIIGPPRTIYENRIYSLKIECGPKYPEAPPFVRFVTKINMNGVNSSN**  
**GVVDPRAISVLAKWQNSYSIKVVLQELRRLMMSKENMKLPQPPPEGQCYSN**

Native sequence in bold, Start Met is missing  
Protease cleavage Precission site underlined  
Cloning sites BamH1 / SalI

**DNA sequence of insert**  
GGATCCCCAGGAGAGGTTCAAGCGTCTTACCTGAAGTCACAAAGCAAAC  
GAGTGATGAAGGAAGACTTGAACCTAGAAAATTTCACTGCAAAGGAGTAA  
AAGTCCCTCGCAATTTCCGACTGTTGGAAGAACTCGAAGAAGGCCAGAAA  
GGAGTAGGAGATGGCACAGTTAGCTGGGGTCTAGAAGATGACGAAGACAT  
GACACTTACAAGATGGACAGGGATGATAATTGGGCCTCCAAGAACAATTT  
ATGAAAACCGAATATACAGCCTTAAAATAGAATGTGGACCTAAATACCCA  
GAAGCACCCCCCTTGTAAAGATTTGTAACAAAAATTAATATGAATGGAGT  
AAATAGTTCTAATGGAGTGGTGGACCCAAGAGCCATATCAGTGCTAGCAA  
AATGGCAGAATTCATATAGCATCAAAGTTGTCTGCAAGAGCTTCGGCGC  
CTAATGATGTCTAAAGAAAATATGAAACTCCCTCAGCCGCCCGAAGGACA  
GTGTTACAGCAATTAAGTCGAC