

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2V1

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

Clone number:- DU20496

Source:- BL21 recombinant

Tag:- cleaved from N-terminal His₆-tag

Purification method:- Ni⁺⁺-NTA-Sepharose, protease treatment, SEC

Expression level:- 2mg/L

Calculated molecular mass:-

Monoisotopic 19461 Da

Average Mass 19474 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.19

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

His-UBE2V1

Protein UBE2V1
Synonyms Uev1A, CIR1, CROC1
Clone Number DU20496
Species Human
Accession Number Protein: NP_068823 DNA: NM_021988

Tags N-terminal His₆ tag

Aminoacid sequence of the expressed protein **GPGSPGEVQASYLKSQSKLSDEGRLEPRKFHCKGVKVPRNFRLLLEELEEG
**QKGVGDGTVSWGLEDDEDMTLTRWTGMIIGPPRTIYENRIYSLKIECGPK
**YPEAPPFVRFVTKINMNGVNSSNGVVDPRAISVLAKWQNSYSIKVVLQEL
RRLMMSKENMKLPQPPEGQCYSN******

Native sequence in bold, Start Met is missing

Protease cleavage Thrombin site underlined

Cloning sites BamH1 / Sal1

DNA sequence of insert **GGATCCCCAGGAGAGGTTCAAGCGTCTTACCTGAAGTCACAAAGCAAACT
GAGTGATGAAGGAAGACTTGAACCTAGAAAATTTCACTGCAAAGGAGTAA
AAGTCCCTCGCAATTTCCGACTGTTGGAAGAAGCTCGAAGAAGGCCAGAAA
GGAGTAGGAGATGGCACAGTTAGCTGGGGTCTAGAAGATGACGAAGACAT
GACACTTACAAGATGGACAGGGATGATAATTGGGCCCTCAAGAACAATTT
ATGAAAACCGAATATACAGCCTTAAAATAGAATGTGGACCTAAATACCCA
GAAGCACCCCTTTGTAAGATTTGTAACAAAAATTAATATGAATGGAGT
AAATAGTTCTAATGGAGTGGTGGACCCAAGAGCCATATCAGTGCTAGCAA
AATGGCAGAATTCATATAGCATCAAAGTTGTCTGCAAGAGCTTCGGCGC
CTAATGATGTCTAAAGAAAATATGAAACTCCCTCAGCCGCCCGAAGGACA
GTGTTACAGCAATTAAGTCGAC**