

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2N

<u>Enzyme description:-</u>	UBE2N
<u>Clone number:-</u>	DU15705
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	cleaved from N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -NTA-Sepharose, protease treatment
<u>Expression level:-</u>	7mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	17294 Da
Average Mass	17303Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.4
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT	
<u>Storage temperature:-</u>	-80°C

Assay:-

Production of free K63 linked Ub-chains with Ubiquitin, UBE1 and UBE2V1 in the presence of Mg-ATP

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Clone Data Sheet

UBE2N

Protein UBE2N
Synonyms Ubc13, BLU
Clone Number DU15705
Species Human
Accession Number Protein: P61088 DNA: NM_003348
Tags N-terminal His₆ tag (cleaved)
Aminoacid sequence of the expressed protein **GPGSAGLPRRI**I**KET**Q**RLLAEPV**P**G**I**KAEPDESNARYFHVVIAG**P**QDS**P**F**E**G
GTFKLEL**F**L**P**EE**Y**P**M**AAPK**V**RF**M**TK**I**YHPNVDK**L**GRICLDILKDKWSPAL**Q****I**
RTVLL**S**I**Q**ALL**S**APNP**D**D**P**L**A**ND**V**AE**Q**WKT**N**EA**Q**AI**E**T**A**RAW**T**RL**Y**AM**N**I
Native sequence in bold, Start Met is missing
Protease cleavage Prescission site underlined
Cloning sites BamH1 / EcoRI**

DNA sequence of insert
GGATCCGCCGGGCTGCCCGCAGGATCATCAAGGAAACCCAGCGTTTGCT
GGCAGAACCAGTTCCTGGCATCAAAGCCGAACCAGATGAGAGCAACGCC
GTTATTTTTCATGTGGTCATTGCTGGCCCTCAGGATCCCCCTTTGAGGGA
GGGACTTTTAAACTTGAAC**T**ATTCCTTCCAGAAGAATACCCAATGGCAGC
CCCTAAAGTACGTTTCATGACCAAAATTTATCATCCTAATGTAGACAAGT
TGGGAAGAATATGTTTAGATATTTTGAAAGATAAGTGGTCCCCAGCACTG
CAGATCCGCACAGTTC**T**GCTATCGATCCAGGCCTTGTTAAGT**G**CTCCCAA
TCCAGATGATCCATTAGCAAATGATGTAGCGGAGCAGTGGAAAGACCAACG
AAGCCCAAGCCATAGAAACAGCTAGAGCATGGACTAGGCTATATGCCATG
AATAATATTTAAGAATTC