

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2T

<u>Enzyme description:-</u>	UBE2T 1-197 (full length)
<u>Clone number:-</u>	DU12416
<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>	2mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	22804 Da
Average Mass	22818 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	8.8
<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
<u>Assay:-</u>	
Loading with Ubiquitin and UBE1 in the presence of Mg-ATP. Assay buffer: 50mM HEPES pH 7.5	

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Clone Data Sheet

UBE2T

Protein UBE2T
Synonyms HSPC150, PIG50
Clone Number DU12416
Species Human
Accession Number Protein: NP_054895 DNA: NM_014176

Tags N-terminal His-tag (cleaved)

Aminoacid sequence of the purified protein **GPGSMQRASRLKRELHMLATEPPPGITCWQDKDQMDLRAQILGGANTPY
EKGVFKLEVIIPERYPFEPQIRFLTPITYHPNIDSAGRICLDVLKLPKG
AWRPSLNIATVLTSIQLLMSEPNPDDPLMADISSEFKYNKPAFLKNARQW
TEKHARQKQKADEEEMLDNLPEAGDSRVHNSTQKRKASQLVGIKKFHPD
V**

Native sequence in bold

Protease cleavage Prescission site underlined

Cloning sites BamH1 / NotI

DNA sequence of insert GGATCCATGCAGAGAGCTTCACGTCTGAAGAGAGAGCTGCACATGTTAGC
CACAGAGCCACCCCAGGCATCACATGTTGGCAAGATAAAGACCAAATGG
ATGACCTGCGAGCTCAAATATTAGGTGGAGCCAACACACCTTATGAGAAA
GGTGTTTTTAAGCTAGAAGTTATCATTCTGAGAGGTACCCATTTGAACC
TCCTCAGATCCGATTTCTCACTCCAATTTATCATCCAAACATTGATTCTG
CTGGAAGGATTTGTCTGGATGTTCTCAAATTGCCACCAAAGGTGCTTGG
AGACCATCCCTCAACATCGCAACTGTGTTGACCTCTATTCAGCTGCTCAT
GTCAGAACCCAACCCTGATGACCCGCTCATGGCTGACATATCCTCAGAAT
TTAAATATAATAAGCCAGCCTTCTCAAGAATGCCAGACAGTGGACAGAG
AAGCATGCAAGACAGAAACAAAAGGCTGATGAGGAAGAGATGCTTGATAA
TCTACCAGAGGCTGGTGACTCCAGAGTACACAACCTCAACACAGAAAAGGA
AGGCCAGTCAGCTAGTAGGCATAGAAAAGAAATTCATCCTGATGTTTAG
GCGGCCGC