

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2T

Enzyme description:- His-UBE2T 1-197 (full length)

Clone number:- DU12416

Source:- BL21 recombinant

Tag:- N-terminal His₆-tag

Purification method:- Ni⁺⁺-NTA-Sepharose

Expression level:- 2mg/L

Calculated molecular mass:-

Monoisotopic 24949 Da

Average Mass 24964 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.7

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80°C

Assay:-

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

GST-UBE2T

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

Tags N-terminal His-tag

Aminoacid sequence of the expressed protein **MGSSHHHHHHSSGLEVLFOGPGSMQRASRLKRELHMLATEPPPGITCWQDKDQMDLRAQILGGANTPYEKGVFKLEVIIPERYPFEPPIRFLTPITYHPNIDSAGRICLDVLKLPKGAWRPSLNIATVLTSIQLLMSEPNPDDPLMADISSEFKYNKPAFLKNARQWTEKHARQKQKADEEEMLDNLPEAGDSRVHNS**
TQKRKASQLVGLIEKKFHPDV

Native sequence in bold

Protease cleavage Prescission site underlined

Cloning sites BamH1 / NotI

DNA sequence of insert
GGATCCATGCAGAGAGCTTCACGTCTGAAGAGAGAGCTGCACATGTTAGC
CACAGAGCCACCCCAGGCATCACATGTTGGCAAGATAAAGACCAAATGG
ATGACCTGCGAGCTCAAATATTAGGTGGAGCCAACACACCTTATGAGAAA
GGTGTTTTTTAAGCTAGAAGTTATCATTCCTGAGAGGTACCCATTTGAACC
TCCTCAGATCCGATTTCTCACTCCAATTTATCATCCAAACATTGATTCTG
CTGGAAGGATTTGTCTGGATGTTCTCAAATTGCCACAAAAGGTGCTTGG
AGACCATCCCTCAACATCGCAACTGTGTTGACCTCTATTCAGCTGCTCAT
GTCAGAACCCAACCCTGATGACCCGCTCATGGCTGACATATCCTCAGAAT
TTAAATATAATAAGCCAGCCTTCTCAAGAATGCCAGACAGTGGACAGAG
AAGCATGCAAGACAGAAACAAAAGGCTGATGAGGAAGAGATGCTTGATAA
TCTACCAGAGGCTGGTGACTCCAGAGTACACAACCAACACAGAAAAGGA
AGGCCAGTCAGCTAGTAGGCATAGAAAAGAAATTCATCCTGATGTTTAG
GCGGCCGC