

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2S

Enzyme description:- GST-UBE2S 2-222 (full length w/o start Met)

Clone number:- DU4205

Source:- BL21 recombinant

Tag:- N-terminal GST-tag

Purification method:- GSH-Sepharose

Expression level:- 5mg/L

Calculated molecular mass:-

Monoisotopic 50536 Da

Average Mass 50506 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.87

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

His-UBE2S

Protein UBE2S (2-222)
Synonyms E2-EPF, EPF5
Clone Number DU4205
Species Human
Accession Number Protein: NP_055316, DNA: NM_014501
Tags N-terminal GST-tag

Aminoacid sequence of the expressed protein **MSPILGYWKIKGLVQPTRLLEYLEEKYEHLIERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIRYIADKHNMLGGCPKERAESMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSNSNVENLPPHIIRLVYKEV**TTLTADPPDGIKVF**PNEEDL**TDLQVTIEGPEGTPYAGGLFRMKLLGKDFPASPPKGYFLTKIFHPNVGANGEICVNVLKRDWTAELGIRHVLLTIKLLIHPNPESALNEEAGRLLLENYEEYAARALLTEIHGGAGGPPSGRAEAGRALASGTEASSTDPGAPGGPGGAEGPMAKKHAGERDKKLAAKKTKDKKRALRL****

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

DNA sequence of insert GGATCCAACTCCAACGTGGAGAACCTACCCCCGCACATCATCCGCCTGGTGTACAAGGAGGTGACGACACTGACCGCAGACCCACCCGATGGCATCAAGGTCTTTCCCAACGAGGAGGACCTACCGACCTCCAGGTACCCATCGAGGGCCCTGAGGGGACCCATATGCTGGAGGTCTGTTCCGCATGAAACTCCTGCTGGGGAAGGACTTCCTGCCTCCCCACCCAAGGGCTACTTCCTGACCAAGATCTTCCACCCGAACGTGGGCGCCAATGGCGAGATCTGCGTCAACGTGCTCAAGAGGACTGGACGGCTGAGCTGGGCATCCGACACGTACTGCTGACCATCAAGTGCCTGCTGATCCACCCTAACCCGAGTCTGCACTCAACGAGGAGGCGGGCCGCTGCTCTTGGAGAACTACGAGGAGTATGCAGCTCGGGCCGTCTGCTCACAGAGATCCACGGGGCGCCGGCGGGCCAGCGGCAGGGCCGAAGCCGGTCGGGCCCTGGCCAGTGGCACTGAAGCTTCCTCCACCGACCCTGGGGCCCCAGGGGGCCGGGAGGGGCTGAGGGTCCCATGGCCAAGAAGCATGCTGGCGAGCGGATAAGAAGCTGGCGGCCAAGAAAAAGACGGACAAGAAGCGGGCGCTGCGGCGGCTGTAGGCGGCCG