Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2Z

Enzyme description:- His-UBE2Z 1-354 (full length)

Clone number:- DU20121

Source:- BL21 recombinant

Tag:- N-terminal His_6-tag

Purification method:- Ni^{++}-NTA-Sepharose

Expression level:- 7mg/L

Calculated molecular mass:-

Monoisotopic	40628 Da
Average Mass	40653 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.88

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80ºC

Assay:-

Loading with Ubiquitin and UBE1 in the presence of Mg-ATP
Loading buffer: 50mM HEPES pH 7.5

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Clone Data Sheet

His-UBE2Z

Protein	UBE2Z
Synonyms	USE1
Clone Number	DU20121
Species	Human
Accession Number	Protein: Q9H832 DNA: NM_023079.3
Tags	N-terminal His-tag
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSGLEVLFQGPGS**MAESPTEEAATAGAGAAGPGASSVAGV VGVSGSGGGFGPPFLPDVWAAAAAAGGAGGPGSGLAPLPGLPPSAAAHGA ALLSHWDPTLSSDWDGERTAPQCLLRIKRDIMSIYKEPPPGMFVVPDTVD MTKIHALITGPFDTPYEGGFFLFVFRCPPDYPIHPPRVKLMTTGNNTVRF NPNFYRNGKVCLSILGTWTGPAWSPAQSISSVLISIQSLMTENPYHNEPG FEQERHPGDSKNYNECIRHETIRVAVCDMMEGKCPCPEPLRGVMEKSFLE YYDFYEVACKDRLHLQGQTMQDPFGEKRGHFDYQSLLMRLGLIRQKVLER LHNENAEMDSDSSSSGTETDLHGSLRV**
Native sequence	in bold
Protease cleavage	Prescission protease site underlined
Cloning sites	BamH1 / Not1
DNA sequence of insert	GGATCCATGGCGGAGAGTCCGACTGAGGAGGCGGCAACGGCGGGCGCCGG GGCGGCGGGCCCCGGGGCGAGCAGCGTTGCTGGTGTTGTTGGCGTTAGCG GCAGCGGCGGCGGGTTCGGGCCGCCTTTCCTGCCGGATGTGTGGGCGGCG GCGGCGGCAGCGGGCGGGGCCGGGGGCCCGGGGAGCGGCCTGGCTCCGCT GCCCGGGCTCCCGCCCTCAGCCGCTGCCCACGGGGCCGCGCTGCTTAGCC ACTGGGACCCCACGCTCAGCTCCGACTGGGACGGCGAGCGCACCGCGCCG CAGTGTCTACTCCGGATCAAGCGGGATATCATGTCCATTTATAAGGAGCC TCCTCCAGGAATGTTCGTTGTACCTGATACTGTTGACATGACTAAGATTC ATGCATTGATCACAGGCCCATTTGACACTCCTTATGAAGGGGGTTTCTTC CTGTTCGTGTTTCGGTGTCCGCCCGACTATCCCATCCACCCACCTCGGGT CAAACTGATGACAACGGGCAATAACACAGTGAGGTTTAACCCCAACTTCT ACCGCAATGGGAAAGTCTGCTTGAGTATTCTAGGTACATGGACTGGACCT GCCTGGAGCCCAGCCCAGAGCATCTCCTCAGTGCTCATCTCTATCCAGTC CCTGATGACTGAGAACCCCTATCACAATGAGCCCGGCTTTGAACAGGAGA GACATCCAGGAGACAGCAAAAACTATAATGAATGTATCCGGCACGAGACC ATCAGAGTTGCAGTCTGTGACATGATGGAAGGAAAGTGTCCCTGTCCTGA ACCCCTACGAGGGGTGATGGAGAAGTCCTTTCTGGAGTATTACGACTTCT ATGAGGTGGCCTGCAAAGATCGCCTGCACCTTCAAGGCCAAACTATGCAG GACCCTTTTGGAGAGAAGCGGGGCCACTTTGACTACCAGTCCCTCTTGAT GCGCCTGGGACTGATACGTCAGAAAGTGCTGGAGAGGCTCCATAATGAGA ATGCAGAAATGGACTCTGATAGCAGTTCATCTGGGACAGAGACAGACCTT CATGGGAGCCTGAGGGTTTAGGCGGCCGC