Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-UBE2R2

Enzyme description:- UBE2R2

Clone number:- DU20557

Source:- BL21 recombinant

Tag:- N-terminal His_6-tag

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

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic	30690 Da
Average Mass	30709 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.56

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

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Clone Data Sheet

His-UBE2R2

Protein	UBE2R2
Synonyms	Ubc3B, Cdc34B
Clone Number	DU20557
Species	Human
Accession Number	Protein: Q712K3 DNA: NM_017811
Tags	N-terminal His_6-tag
Aminoacid sequence of the expressed protein	MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS**MAQQQMTSSQKALMLE LKSLQEEPVEGFRITLVDESDLYNWEVAIFGPPNTLYEGGYFKAHIKFPI DYPYSPPTFRFLTKMWHPNIYENGDVCISILHPPVDDPQSGELPSERWNP TQNVRTILLSVISLLNEPNTFSPANVDASVMFRKWRDSKGKDKEYAEIIR KQVSATKAEAEKDGVKVPTTLAEYCIKTKVPSNDNSSDLLYDDLYDDDID DEDEEEEDADCYDDDDSGNEES**
Native sequence	in bold
Protease cleavage	Thrombin site underlined
Cloning sites	BamH1 / Not1

DNA sequence of the insert

GGATCCATGGCCCAGCAGCAGATGACCAGCTCGCAGAAGGCCCTGATGCT
CGAGCTGAAATCCCTGCAGGAGGAACCGGTGGAGGGCTTCCGGATCACCC
TGGTGGACGAGTCCGACCTCTACAACTGGGAGGTGGCCATCTTCGGACCC
CCCAACACCCTCTACGAAGGCGGCTACTTCAAGGCGCATATTAAATTTCC
TATTGACTACCCCTATTCACCACCTACCTTCAGATTCTTGACCAAAATGT
GGCACCCCAACATTTATGAGAATGGAGATGTATGCATTTCGATTCTTCAT
CCGCCTGTAGATGACCCACAGAGTGGAGAACTGCCTTCTGAAAGGTGGAA
TCCTACTCAGAATGTGAGGACTATCCTATTAAGTGTAATCTCACTGCTTA
ATGAGCCCAACACCTTCTCCCCAGCCAATGTCGATGCTTCAGTTATGTTC
AGGAAATGGAGAGACAGTAAAGGAAAAGACAAAGAATATGCTGAAATTAT
TAGGAAACAAGTTTCAGCCACTAAGGCCGAAGCAGAGAAGGATGGAGTGA
AGGTCCCCACAACCCTGGCGGAATACTGCATCAAAACTAAAGTGCCTTCC
AATGACAACAGCTCAGATTTGCTTTACGACGACTTGTATGATGACGACAT
TGATGATGAAGATGAGGAGGAGGAAGATGCCGACTGTTATGATGATGATG
ATTCTGGGAATGAGGAGTCGTGAGCGGCCGC