Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of UBE2I

Enzyme description:- UBE2I

Clone number:- DU3908

Source:- BL21 recombinant

Tag:- cleaved from N-terminal GST-tag

Purification method:- GSH-Sepharose, protease treatment

Expression level:- 2mg/L

Calculated molecular mass:-

Monoisotopic	18406 Da
Average Mass	18417 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 9.4

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80ºC

Assay:-

Loading assay with SUMO and SAE1/SAE2 in the presence of Mg-ATP

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Clone Data Sheet

UBE2I

Protein	UBE2I
Synonyms	Ubc9
Clone Number	DU3908
Species	Human
Accession Number	Protein: NP_003336
Tags	N-terminal GST-tag (cleaved)
Aminoacid sequence of the expressed protein	GPLGS**MSGIALSRLAQERKAWRKDHPFGFVAVPTKNPDGTMNLMNWECAIPG KKGTPWEGGLFKLRMLFKDDYPSSPPKCKFEPPLFHPNVYPSGTVCLSILEE DKDWRPAITIKQILLGIQELLNEPNIQDPAQAEAYTIYCQNRVEYEKRVRAQ AKKFAPS**
Native sequence	in bold
Protease cleavage	Prescission protease site underlined
Cloning sites	BamH1 / Not1

DNA sequence of insert

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GGATCCATGTCGGGGATCGCCCTCAGCAGACTCGCCCAGGAGAGGAAAGC
ATGGAGGAAAGACCACCCATTTGGTTTCGTGGCTGTCCCAACAAAAAATC
CCGATGGCACGATGAACCTCATGAACTGGGAGTGCGCCATTCCAGGAAAG
AAAGGGACTCCGTGGGAAGGAGGCTTGTTTAAACTACGGATGCTTTTCAA
AGATGATTATCCATCTTCGCCACCAAAATGTAAATTCGAACCACCATTAT
TTCACCCGAATGTGTACCCTTCGGGGACAGTGTGCCTGTCCATCTTAGAG
GAGGACAAGGACTGGAGGCCAGCCATCACAATCAAACAGATCCTATTAGG
AATACAGGAACTTCTAAATGAACCAAATATCCAAGACCCAGCTCAAGCAG
AGGCCTACACGATTTACTGCCAAAACAGAGTGGAGTACGAGAAAAGGGTC
CGAGCACAAGCCAAGAAGTTTGCGCCCTCATAAGCGGCCGC
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