

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2I

<u>Enzyme description:-</u>	GST-UBE2I
<u>Clone number:-</u>	DU3908
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	N-terminal GST-tag
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	10mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	44801 Da
Average Mass	44829 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.24
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT	
<u>Storage temperature:-</u>	-80°C

Assay:-

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

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Clone Data Sheet

GST-UBE2I

<u>Protein</u>	UBE2I
<u>Synonyms</u>	Ubc9
<u>Clone Number</u>	DU3908
<u>Species</u>	Human
<u>Accession Number</u>	Protein: NP_003336
<u>Tags</u>	N-terminal GST-tag
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPHYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSM SGIALSRLAQERKAWRKDHPFGFVAVPTKNPDGTMNLMNWECAIPGKKGTPWEGGLFKLRMLFKDDYPSSPPKCKFEPPLFHPNVYPSGTVCLSILEEDKDWRPAITIKQILLGIQEL LNEPNIQDPAQAEAYTIYCQNRVEYEKRVRAQAKKFAPS
Native sequence	in bold
Protease cleavage	Prescission protease site underlined
Cloning sites	BamH1 / NotI
<u>DNA sequence of insert</u>	GGATCCATGTCTGGGGATCGCCCTCAGCAGACTCGCCCAGGAGAGGAAAGCATGGAGGAAAGACCACCCATTTGGTTTCGTGGCTGTCCCAACAAAAAATCCCGATGGCACGATGAACCTCATGAAC TGGGAGTGCGCCATTCCAGGAAAGAAAGGGACTCCGTGGGAAGGAGGCTTGTTTAAACTACGGATGCTTTTCAAAGATGATTATCCATCTTCGCCACCAAATGTAAATTCGAACCACCATTATTTCACCCGAATGTGTACCCTTCGGGGACAGTGTGCCTGTCCATCTTAGAGGAGGACAAGGACTGGAGGCCAGCCATCACAATCAAACAGATCCTATTAGGAATACAGGAACTTCTAAATGAACCAAATATCCAAGACCCAGCTCAAGCAGAGGCCTACACGATTTACTGCCAAAACAGAGTGGAGTACGAGAAAAGGGTCGAGCACAAGCCAAGAAGTTTGCGCCCTCATAAGCGGCCGC