Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2B

Enzyme description:- GST-UBE2B (2-152)

Clone number:- DU4204

Source:- human recombinant

Tag:- N-terminal GST-tag

Purification method:- GSH-Sepharose

Expression system:- *E.coli*

Calculated molecular mass:-

Monoisotopic	43977 Da
Average Mass	44004 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.43

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

GST-UBE2B

Protein	GST-UBE2B (2-152)
Synonyms	ubiquitin-conjugating enzyme E2B, Rad 6 homolog B
Clone Number	DU4204
Species	Human
Accession Number	Protein: NP_003328 DNA: NM_003337
Tags	N-terminal GST-tag
Aminoacid sequence of the expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**STPARRRLMRDFKRLQEDP PVGVSGAPSENNIMQWNAVIFGPEGTPFEDGTFKLVIEFSEEYPNKPPTV RFLSKMFHPNVYADGSICLDILQNRWSPTYDVSSILTSIQSLLDEPNPNS PANSQAAQLYQENKREYEKRVSAIVEQSWNDS**
Native sequence	Full length, but start Met missing
Protease cleavage	Prescission site underlined
Cloning sites	BamH1 / EcoR1
DNA sequence of insert	ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC TCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA GTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTG GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAAC TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCG AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC AATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCTCGACCC CGGCCCGGAGGAGGCTCATGCGGGATTTCAAGCGGTTACAAGAGGACCCA CCTGTGGGTGTCAGTGGCGCACCATCTGAAAACAACATCATGCAGTGGAA TGCAGTTATATTTGGACCAGAAGGGACACCTTTTGAAGATGGTACTTTTA AACTAGTAATAGAATTTTCTGAAGAATATCCAAATAAACCACCAACTGTT AGGTTTTTATCCAAAATGTTTCATCCAAATGTGTATGCTGATGGTAGCAT ATGTTTAGATATCCTTCAGAATCGATGGAGTCCAACATATGATGTATCTT CTATCTTAACATCAATTCAGTCTCTGCTGGATGAACCGAATCCTAACAGT CCAGCCAATAGCCAGGCAGCACAGCTTTATCAGGAAAACAAACGAGAATA TGAGAAAAGAGTTTCGGCCATTGTTGAACAAAGCTGGAATGATTCATAAG CGGCCAAGGGCGAATTCC