Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-OTUB1 [C91S]

Enzyme description:- GST-OTUB1

Clone number:- DU20873

Source:- BL21 Recombinant

Tag:- N-terminal GST tag

Purification method:- GSH sepharose

Expression level:- 3 mg/L

Calculated molecular mass:-

Monoisotopic	58055 Da
Average Mass	58090 Da

[cycsteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.08

Purity:- 90%

Enzyme storage buffer:-

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

Storage temperature:- -80ºC

Assay:-

Ub-Rho110-Gly cleavage assay monitored by Ex/Em 485/535 nm

Assay buffer:-

40 mM Tris pH 7.5, 100 mM NaCl, 5 mM DTT, 0.01% Triton X-100, 0.005% Ovalbumin, 0.5 μM Ub-Rho110-Gly

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Clone Data Sheet

GST-OTUB1 [C91S]

Protein	GST-OTUB1 [C91S]
Synonyms	
Clone Number	DU20873
Species	Human
Accession Number	Protein: Q96FW1 DNA: NM_017670
Tags	N-terminal GST tag
Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGV SRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL DVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGG GDHPPKSDLEVLFQGPLGS**MAAEEPQQQKQEPLGSDSEGVNCLAYDEAIMAQQ DRIQQEIAVQNPLVSERLELSVLYKEYAEDDNIYQQKIKDLHKKYSYIRKTRP DGNSFYRAFGFSHLEALLDDSKELQRFKAVSAKSKEDLVSQGFTEFTIEDFHN TFMDLIEQVEKQTSVADLLASFNDQSTSDYLVVYLRLLTSGYLQRESKFFEHF IEGGRTVKEFCQQEVEPMCKESDHIHIIALAQALSVSIQVEYMDRGEGGTTNP HIFPEGSEPKVYLLYRPGHYDILYK**
Native sequence	in bold
Protease cleavage	Precission site underlined
Cloning sites	BamH1 / Not1
DNA sequence of insert	GGATCCATGGCGGCGGAGGAACCTCAGCAGCAGAAGCAGGAGCCGCTGGGCAG CGACTCCGAAGGTGTTAACTGTCTGGCCTATGATGAAGCCATCATGGCTCAGC AGGACCGAATTCAGCAAGAGATTGCTGTGCAGAACCCTCTGGTGTCAGAGCGG CTGGAGCTCTCGGTCCTATACAAGGAGTATGCTGAAGATGACAACATCTATCA ACAGAAGATCAAGGACCTCCACAAAAAGTACTCGTACATCCGCAAGACCAGGC CTGACGGCAACAGTTTCTATCGGGCTTTCGGATTCTCCCACTTGGAGGCACTG CTGGATGACAGCAAGGAGTTGCAGCGGTTCAAGGCTGTGTCTGCCAAGAGCAA GGAAGACCTGGTGTCCCAGGGCTTCACTGAATTCACAATTGAGGATTTCCACA ACACGTTCATGGACCTGATTGAGCAGGTGGAGAAGCAGACCTCTGTCGCCGAC CTGCTGGCCTCCTTCAATGACCAGAGCACCTCCGACTACCTTGTGGTCTACCT GCGGCTGCTCACCTCGGGCTACCTGCAGCGCGAGAGCAAGTTCTTCGAGCACT TCATCGAGGGTGGACGGACTGTCAAGGAGTTCTGCCAGCAGGAGGTGGAGCCC ATGTGCAAGGAGAGCGACCACATCCACATCATTGCGCTGGCCCAGGCCCTCAG CGTGTCCATCCAGGTGGAGTACATGGACCGCGGCGAGGGCGGCACCACCAATC CGCACATCTTCCCTGAGGGCTCCGAGCCCAAGGTCTACCTTCTCTACCGGCCT GGACACTACGATATCCTCTACAAATAGGCGGCCGC