

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-OTUB1

<u>Enzyme description:-</u>	GST-OTUB1
<u>Clone number:-</u>	DU19741
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal GST tag
<u>Purification method:-</u>	GSH sepharose
<u>Expression level:-</u>	3 mg/L

Calculated molecular mass:-

Monoisotopic	58071 Da
Average Mass	58106 Da
[cysteines 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:- -80°C

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

<u>Protein</u>	GST-OTUB1
<u>Synonyms</u>	
<u>Clone Number</u>	DU19741
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q96FW1 DNA: NM_017670
<u>Tags</u>	N-terminal GST tag
<u>Amino acid sequence of expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGV SRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL DVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQATFGG GDHPPKSDLEVLFOGPLGSMAEEPQOQKQEPLGSDSEGVNCLAYDEAIMAQO DRIQOEIAVQNPLVSERLELSVLYKEYAEDDNIYQOKIKDLHKKYSYIRKTRP DGNCFYRAFGFSHLEALLDDSKELQRFKAVSAKSKEDLVSQGFTEFTIEDFHN TFMDLIEQVEKQTSVADLLASFNDQSTSDYLVVYLRLLTSGYLQRESKFFEHF IEGGRTVKEFCQQVEPMCKESDHIIALAQALSSVSIQVEYMDRGEGGTTNP HIFPEGSEPKVYLLYRPGHYDILYK</p>
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	BamH1 / NotI
<u>DNA sequence of insert</u>	<p>GGATCCATGGCGGCGGAGGAACCTCAGCAGCAGAAGCAGGAGCCGCTGGGCAG CGACTCCGAAGGTGTTAACTGTCTGGCCTATGATGAAGCCATCATGGCTCAGC AGGACCGAATTCAGCAAGAGATTGCTGTGCAGAACCCTCTGGTGTGAGAGCGG CTGGAGCTCTCGGTCCTATACAAGGAGTATGCTGAAGATGACAACATCTATCA ACAGAAGATCAAGGACCTCCACAAAAAGTACTCGTACATCCGCAAGACCAGGC CTGACGGCAACTGTTTCTATCGGGCTTTCGGATTCTCCCACTTGGAGGCACTG CTGGATGACAGCAAGGAGTTGCAGCGGTTCAAGGCTGTGTCTGCCAAGAGCAA GGAAGACCTGGTGTCCCAGGGCTTCACTGAATTCACAATTGAGGATTTCCACA ACACGTTTATGGACCTGATTGAGCAGGTGGAGAAGCAGACCTCTGTGCGCCGAC CTGCTGGCCTCCTTCAATGACCAGAGCACCTCCGACTACCTTGTGGTCTACCT GCGGCTGCTCACCTCGGGCTACCTGCAGCGCGAGAGCAAGTTCTTTCGAGCACT TCATCGAGGGTGGACGGACTGTCAAGGAGTTCTGCCAGCAGGAGGTGGAGCCC ATGTGCAAGGAGAGCGACCACATCCACATCATTGCGCTGGCCCAGGCCCTCAG CGTGTCCATCCAGGTGGAGTACATGGACCGCGGCGAGGGCGGCACCACCAATC CGCACATCTTCCCTGAGGGCTCCGAGCCCAAGGTCTACCTTCTCTACCGGCCT GGACACTACGATATCTCTACAAATAGGCGGCCGC</p>