

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-OTULIN

<u>Enzyme description:-</u>	GST-OTULIN
<u>Clone number:-</u>	DU43487
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH-Sepharose
<u>Expression level:-</u>	3 mg/L

Calculated molecular mass:-

Monoisotopic	66898 Da
Average Mass	66939 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.66

Purity:- 95%

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-OTULIN

<u>Protein</u>	GST-OTULIN
<u>Synonyms</u>	fam105B
<u>Clone Number</u>	DU43487
<u>Species</u>	Human
<u>Accession Number</u>	Protein: Q96BN8 DNA: NM_138348.4
<u>Tags</u>	N-terminal
<u>Amino acid sequence of expressed protein</u>	MSPILGYWKIKGLVQPTRLRLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKL TQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDENLYFQGGSMSRGTMPQPEAWPGASCAETP AREAAATARDGGKAAASGQPRPEMQCPAEHEEDMYRAADEIEKEKELLIH ERGASEPRLSVAPEMD IMDYCKKEWRGNTQKATCMKMGYEEVSQKFTSIR RVRGDN YCALRATL FQAMSQAVGLPPWLQDPELMLLPEKLI SKYNWIKQW KLGLKFDGKNEDLVDKI KESLTLRKKWAGLAEMRTAEARQIACDELFTN EAEYSLYEAVKFLMLNRAIELYNDKEGKEVPPFSVLLFARDTSNDPGQ LLRNHLNQVGHTGGLEQVEMFLLAYAVRHTIQVYRLSKYNTTEFITVYPT DPPKDWPVVTLIAEDDRHYNIPVRVCEETSL
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	TEV site underlined
<u>Cloning sites</u>	BamH1/Not1
<u>DNA sequence of insert</u>	GGATCCATGAGTCGGGGGACTATGCCCCAGCCCGAAGCGTGGCCAGGCGC GAGCTGCGCCGAGACGCCGGCGGGAGGCGGCCACGGCGGGGACG GCGGGAAGGCGGCGGCCAGCGGGCAGCCGCGGCCCGAGATGCAGTGCCCCG GCCGAGCATGAGGAGGACATGTACCGTGCTGCAGATGAAATAGAAAAGGA GAAAGAATTGCTTATACATGAAAGAGGGGCATCAGAACCAGATTAAGCG TAGCTCCTGAAATGGATATCATGGACTACTGCAAAAAGAATGGAGAGGA AATACACAGAAAGCAACGTGTATGAAAATGGGCTATGAAGAGGTTTCTCA GAAGTTCACCTCCATACGGCGAGTCCGTGGTGATAATTACTGTGCACTGA GGGCCACGCTGTTCCAGGCCATGAGCCAGGCTGTGGGGCTGCCGCCCTGG CTGCAGGACCCGAGCTCATGCTGTTACCAGAAAACTCATAAGCAAATA CAACTGGATCAAGCAATGGAACTTGGACTGAAATTTGATGGGAAGAATG AGGACCTGGTTGATAAAATTAAGAGTCCCTTACTCTGCTGAGGAAGAAG TGGGCAGGCTTGGCTGAAATGAGAACTGCTGAAGCAAGACAGATAGCTTG TGATGAACTATTACAAATGAGGCGGAGGAATATAGCCTCTATGAAGCTG TAAAATTTCTAATGCTAAACAGAGCCATTGAACTATATAATGATAAAGAG AAAGGAAAGGAAGTACCATTTTTCTCTGTGCTTCTGTTTGCTCGGGACAC ATCAAAATGACCCAGGACAGCTTCTGAGGAACCACCTCAACCAGGTGGGAC ACACTGGTGGTCTTGAACAGGTTGAAATGTTCTTCTTGCCTATGCTGTG CGCCACACCATCCAGGTGTACCGGCTCTCCAAGTACAACACGGAAGAATT CATCACAGTCTACCCACCGACCCACCCAAGGACTGGCCAGTGGTAACGC TCATTGCTGAGGACGATCGGCACTATAACATCCCCGTCAGAGTGTGTGAG GAGACCAGTCTATGAGCGGCCGC