

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of GST-OTUB2**

<b><u>Enzyme description:-</u></b>	GST-OTUB2
<b><u>Clone number:-</u></b>	DU32795
<b><u>Source:-</u></b>	BL21 Recombinant
<b><u>Tag:-</u></b>	N-terminal GST tag
<b><u>Purification method:-</u></b>	GSH sepharose
<b><u>Expression level:-</u></b>	3 mg/L

#### **Calculated molecular mass:-**

Monoisotopic	54002 Da
Average Mass	54035 Da
[cysteines reduced, methionines have not been oxidised]	

**Theoretical pI:-** 5.92

**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-OTUB2**

<b><u>Protein</u></b>	GST-OTUB2
<b><u>Synonyms</u></b>	OTB2, OTU2
<b><u>Clone Number</u></b>	DU32795
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q96DC9 DNA: NM_023112.3
<b><u>Tags</u></b>	N-terminal GST tag
<b><u>Amino acid sequence of expressed protein</u></b>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQATFGGGDHPPKSDLEVLFOGPLGSMSETSFNLI <b>SEKCDILSILRDHPENRIYRRKIEELSKRFTAIRKTKGDGNCFYRALGYSYLESLLGKSR</b> E <b>IFKFKERV</b> LQTPNDLLAAG <b>FE</b> EHKFRNFFNAFY <b>SVVELVEKDGSVSSLLKVFNDQSASDHI</b> VQFLRLL <b>TS</b> AFIRNRADFFRHF <b>IDEEMDIKDFCTHEVEPMATECDHIQITALSQALSIALQVEYVDEMDTALNHHV</b> FPEAATPSVYLLYKTSY <b>NILYAADKH</b>
<b><u>Native sequence</u></b>	in bold
<b><u>Protease cleavage</u></b>	Precision site underlined
<b><u>Cloning sites</u></b>	BamH1 / NotI
<b><u>DNA sequence of insert</u></b>	GGATCCATGAGTGAAACATCTTTCAACCTAATATCAGAAAAATGTGACAT TCTATCCATTCTTCGGGACCATCCTGAAAACAGGATTTACCGGAGGAAAA TCGAGGAACCTCAGCAAAAGGTTACCGCCATCCGCAAGACCAAAGGGAT GGAACTGCTTCTACAGGGCCTTGGGCTATTCCTACCTGGAGTCCCTGCT GGGAAGAGCAGGGAGATCTTCAAGTTCAAAGAACCGGTA <b>CTGCAGACCC</b> CAAATGACCTTCTGGCTGCTGGCTTTGAGGAGCACAAGTTCAGAACTTC TTCAATGCTTTTACAGTGTGGTGGAACTGGTAGAGAAGGACGGCTCAGT GTCCAGCCTGCTGAAGGTGTTCAACGACCAGAGTGCCCTCGGACCACATCG TGCAGTTCTGCGCCTGCTCACGTCGGCTTCATCAGGAACCGAGCAGAC TTCTTCCGGCACTTCATTGATGAGGAGATGGACATCAAAGACTTCTGCAC TCACGAAGTAGAGCCCATGGCCACGGAGTGTGACCACATCCAGATCACGG CGTTGTGCAGGCCCTGAGCATTGCCCTGCAAGTGGAGTACGTGGACGAG ATGGATACCGCCCTGAACCACCACGTGTTCCCTGAGGCCGCCACCCCTTC CGTTTACCTGCTCTATAAAACATCCCACTACAACATCCTTTATGCAGCCG ATAAACATTGAGCGGCCGC