

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of GST-OTUD6B**

<b><u>Enzyme description:-</u></b>	GST-OTUD6B
<b><u>Clone number:-</u></b>	SC21033
<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>	1 mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	64109 Da
Average Mass	64148 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	6.27
<b><u>Purity:-</u></b>	90%
<b><u>Enzyme storage buffer:-</u></b>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<b><u>Storage temperature:-</u></b>	-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-OTUB6B**

**Protein** GST-OTUB6B  
**Synonyms** DUBA-5  
**Clone Number** SC21140  
**Species** Human  
**Accession Number** Protein: Q8N6M0 DNA: NM\_016023.3  
**Tags** N-terminal GST tag  
**Amino acid sequence of expressed protein** MSPILGYWKIKGLVQPTRLALLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP  
NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAESMLEGAVLDIRYGV  
SRIAYSKDFETLKVDLFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL  
DVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQATFGG  
GDHPPKSDLEVLFOGPLGSMPEPRVVEGWKVP<sup>TSRCRFL</sup>LARVLGYLVV**MEAV**  
**LTEELDEEEQLRRHRKEKKELOAKIQGMKNAVPKNDKRRKQLTEDVAKLEK**  
**EMEQKHREELEQLKLTTKENKIDSVAVNISNLVLENQPPRISKAQKRREKKA**  
**LEKEREERIAEAEIENLTGARHMESEKLAQILAAQLEIKQIPSDGHCMYKAI**  
**EDQLKEKDCALTVVALRSQTAEYMQSHVEDFLPFLTNPNTGDMYTPEEFQKYC**  
**EDIVNTAAWGGQLELRALSHILOTPIEIIQADSPPIIVGEEYSKKPLILVYMR**  
**HAYGLGEHNSVTRLVNIVTENCS**  
**Native sequence** in bold  
**Protease cleavage** Precision site underlined  
**Cloning sites** BamH1 / Not1

**DNA sequence of insert** GGATCCATGGAGCCCCGGGTGAGGGTTGAGGGGTGGAAGGTGCCTACTAGCCG  
GTGCAGGTTTCTTCTAGCGCGTGTGCTGGGGTACCTGGTCGTCATGGAGGCGG  
TATTGACCGAAGAGCTTGATGAGGAAGAGCAGCTGCTGAGAAGGCATCGCAA  
GAGAAGAAGGAGTTGCAAGCCAAAATTCAGGGCATGAAGAATGCTGTTCCCAA

GAATGACAAGAAGAGGAGGAAGCAGCTCACCGAAGATGTGGCCAAGTTGGAAA  
AAGAAATGGAACAGAAACATAGAGAGGAACTGGAGCAATTGAAGCTGACTACT  
AAGGAGAATAAGATAGATTCTGTTGCTGTTAACATTTCAAACCTGGTGCTTGA  
GAATCAGCCACCTCGGATATCAAAAGCACAAAAGAGACGGGAAAAGAAAGCTG  
CATTGGAAAAGGAGCGAGAAGAACGGATAGCTGAAGCTGAAATTGAAAACCTA  
ACAGGAGCCAGACATATGGAAAGTGAGAAACCTGCTCAAATATTGGCAGCTAG  
ACAGTTAGAAATTAAACAGATTCCATCTGATGGCCACTGTATGTATAAAGCCA  
TTGAAGATCAACTGAAAGAAAAGGATTGTGCTCTGACTGTGGTTGCCTTGAGA  
AGTCAGACCGCTGAGTATATGCAAAGCCATGTGGAAGACTTTCTGCCATTTTT  
AACAAACCCTAATACAGGAGATATGTATACTCCAGAAGAATTTCAGAAGTACT  
GTGAAGATATTGTAAACACAGCTGCATGGGGAGGTCAGCTTGAGCTAAGAGCT  
CTGTCTCACATTTTACAAACACCAATAGAGATAATACAGGCAGATTCTCCTCC  
CATTATAGTTGGTGAAGAATATTCAAAAAAACCACTAATACTTGTATATATGA  
GACATGCATATGGCTTAGGAGAACATTATAATTCGGTTACACGGTTGGTAAAC  
ATAGTTACTGAAAATTGCAGCTAAGCGGCCG