

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of His-OTUD3**

<b><u>Enzyme description:-</u></b>	His-OTUD3
<b><u>Clone number:-</u></b>	DU21336
<b><u>Source:-</u></b>	BL21 Recombinant
<b><u>Tag:-</u></b>	N-terminal His <sub>6</sub> -tag
<b><u>Purification method:-</u></b>	Ni <sup>++</sup> -Sephrose
<b><u>Expression level:-</u></b>	1.5 mg/L

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

Monoisotopic	47539 Da
Average Mass	47566 Da
[cysteines reduced, methionines have not been oxidised]	

**Theoretical pI:-** 8.89

**Purity:-** 80 %

#### **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**

**His-OTUD3**

**Protein** His-OTUD3

**Synonyms**

**Clone Number** DU21336

**Species** Human

**Accession Number** Protein: Q5T2D3 DNA: NM\_015207.1

**Tags** N-terminal His<sub>6</sub>-tag

**Amino acid sequence of expressed protein** MGSSHHHHHSSGLEVLFQGPGSMSRKQAAKSRPGSGSRKAEAEERKRDERAAR  
RALAKERRNRPESGGGGCEEEFVSFANQLQALGLKLREVPGDGNCLFRALGD  
QLEGHSRNHLKHRQETVDYMIKQREDFEPFVEDDIPFEKHVASLAKPGTFAGN  
DAIVAFARNHQLNVVIHQLNAPLWQIRGTEKSSVRELHIAYRYGEHYDSVRI  
NDNSEAPAHLOTDFQMLHQDES NKREIKTKGMDSEDDL RDEVEDAVQKVCNA  
TGCSDFNLI VQNL EAENYNIESAI IAVLRMNQGRNNAEENLEPSGRVLKQCG  
PLWEEGGSGARIFGNQGLNEGR TENNKAQASPSEENKANKNQLAKVTNKORRE  
QQWMEKKKQEEERHRHKALES RGS HRDNNRSEAEANTQVTLVKTF AALNI

**Native sequence** in bold

**Protease cleavage** Precision site underlined

**Cloning sites** BamH1/Not1

**DNA sequence of  
insert**

GGATCCATGTCCCGAAAGCAGGCGGGCGAAGAGCCGGCCGGGCAGCGGCAGCCG  
GAAAGCCGAGGCCGAGCGCAAGCGGGACGAGCGGGCGGGCGCCGGGCCCTGG  
CCAAGGAGCGGGCGGAATCGGCCGGAGTCTGGCGGGCGGGCGGGCTGCGAGGAG  
GAGTTCGTCAGCTTCGCCAACCAGCTGCAGGCCCTGGGGCTGAAGCTGCGGGA  
GGTGCCGGGGGACGGCAATTGCTTGTTTCAGAGCTCTTGGTGATCAATTGGAGG  
GACACTCACGAAATCATCTCAAGCACAGACAGGAGACAGTGGACTACATGATA  
AAGCAGCGGGAAGATTTTGAACCCTTTGTAGAAGATGACATTCCTTTTGAGAA  
GCATGTGGCCAGTTTGGCAAAGCCTGGTACTTTTGTGGCAATGATGCAATTG  
TAGCCTTTGCAAGAAATCATCAGTTGAATGTAGTGATTTCATCAACTTAATGCC  
CCTTTGTGGCAGATTCGTGGTACAGAGAAAAGCAGCGTGAGGGAGTTACACAT  
CGCATATCGGTATGGAGAGCACTACGACAGTGTTCCGGAGGATCAATGACAAC  
CAGAGGCACCTGCACATCTCCAGACGGATTTTCAGATGCTTCATCAAGATGAA  
TCAAATAAAAAGAGAAAAGATCAAGACAAAGGGAATGGACTCTGAAGACGACCT  
GAGAGATGAAGTAGAGGATGCTGTCCAGAAAGTTTGTAATGCAACTGGATGTT  
CAGATTTTAATTTAATAGTCCAGAACCTGGAAGCTGAAAATTATAATATTGAA  
TCTGCAATAATTGCCGTGCTTCGGATGAACCAAGGGAAGAGAAATAATGCAGA  
AGAGAATCTTGAGCCCAGTGGTTCGAGTGCTGAAGCAGTGTGGCCCTTTGTGGG  
AGGAGGGTGGCAGTGGTGCCAGAATCTTTGGAAATCAGGGCTTAAATGAAGGC  
AGGACCGAAAACAATAAGGCACAGGCCAGCCCTAGTGAAGAAAACAAAGCAA  
TAAAACCAGCTCGCAAAGGTCACAAACAAACAGAGGCGAGAACAGCAGTGGA  
TGGAGAAGAAGAAGCGGCAGGAGGAGAGGCACCGCCACAAAGCCCTGGAGAGC  
AGAGGTAGCCACAGGGACAATAACAGAAGCGAAGCAGAGGCCGAACACGCAGGT  
CACCTTGGTGAAGACCTTCGCCGCTCTCAACATCTGAGCGGCCGC