

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of GST-OTUD5**

<b><u>Enzyme description:-</u></b>	GST-OTUD5
<b><u>Clone number:-</u></b>	SC20014
<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

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

Monoisotopic	84777 Da
Average Mass	84826 Da
[cysteines reduced, methionines have not been oxidised]	

**Theoretical pI:-** 6.12

**Purity:-** 70%

#### **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-OTUD5

<u>Protein</u>	GST-OTUD5
<u>Synonyms</u>	DUBA
<u>Clone Number</u>	SC20014 (This is probably a rare form of OTUD5)
<u>Species</u>	Human
<u>Accession Number</u>	Protein: AAH98440
<u>Tags</u>	N-terminal GST tag
<u>Amino acid sequence of expressed protein</u>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMTILPKKKPPPDADPGGT GVGGGDRDRDSGVVGARPRASPPPOGPLPGPPGALHRWALAVPPGAVAGP RPQOASPPPCGGPGGGPGGGPDALGAAAAGVGAAGVVVGVGGAVGVGGCC SGPGHSKRRRQAPGVGAVGGGSPEREV GAGYNSEDEYEAAAARI EAMDP ATVEQQEHWF EKALRDKKGFI IKQMKEDGACLFRAVADQVYGDQDMHEV RKHCMDYLMKNADYFSNYVTEDFTTYINRKRKNNCHGNHIEMQAMAEMYN RPVEVYQYSTEPINTFHGIHQNEDEPIRVSYHRNIHYSV VNP NKATIGV GLGLPSFKPGFAEQSLMKNAIKTSEESWIEQQMLEDKKRATDWEATNEAI EEQVARESYLQWLRDQEKQARQVRGSPQPRKASATCSSATAAASSGLEEW TSRSPRQRSSASSPEHPELHAELGMKPPSPGTVLALAKPPSPCAPGTSSQ FSAGADRATSPLVSLYPALECRALIQOMSPSAFGLNDWDDDEILASVLAV SQQEYLD SMKKNK VHRDPPPKS</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>GGATCCATGACTATACTCCCCAAAAGAAGCCGCCGCTCCCGACGCCGACCC CGGCGGCACGGGCGTGGGCGGCGGCATCGCGACCGTGACTCCGGCGTCGTGG GGCCCCGTCCGCGAGCTTCGCCACCGCCTCAAGGCCCGCTACCAGGACCGCCG GGCGCTCTTCATCGCTGGGCGCTGGCCGTGCCGCTGGTGCAGTGGCGGGTCC CCGGCCACAACAGGCTTCTCCACCTCCTTGCGGGGGCCAGGTGGTCCCGGC GCGGTCCCGGCACGCGCTGGGCGCAGCGCGCGGGTGTGGGTGCCCGGGC GTGGTGGTGGGTGTGGGTGGTGCCGTAGGCGTGGGCGGCTGCTGCTCCGGCC TGGGCACAGCAAGCGGCGACGTCAAGCTCCCGGGGTTGGCGCGGTTGGCGGGG GCAGTCCCGAGCGTGAGGAGGTGGGCGCAGGCTACAACAGTGAGGACGAGTAT GAGGCGGCTGCAGCACGCATCGAGGCTATGGACCCTGCCACTGTGAGCAGCA GGAGCATTGGTTTGAAAAGGCCCTACGAGACAAGAAGGGCTTCATCATCAAGC AGATGAAGGAGGATGGCGCTGTCTCTTCCGGGCTGTAGCTGACCAGGTGTAT GGAGACCAGGACATGCATGAGGTTGTGCGAAAGCATTGCATGGACTATCTGAT GAAGAATGCCGACTACTTCTCCAATATGTCACAGAGGACTTTACCACCTACA TTAACAGGAAGCGGAAAAACAATTGCCATGGCAACCACATGAGATGCAGGCC ATGGCAGAGATGTACAACCGTCCTGTGGAGGTGTACCAGTACAGCACAGAACC</p>

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