

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of GST-USP25**

<b><u>Enzyme description:-</u></b>	GST-USP25
<b><u>Clone number:-</u></b>	SC21610
<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>	1.5 mg/L

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

Monoisotopic	148948 Da
Average Mass	149037 Da
[cysteines reduced, methionines have not been oxidised]	

**Theoretical pI:-** 5.28

**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**

**GST-USP25**

<b><u>Protein</u></b>	GST-USP25
<b><u>Synonyms</u></b>	UBP25
<b><u>Clone Number</u></b>	SC21610
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q9UHP3 DNA: NM_013396.3
<b><u>Tags</u></b>	N-terminal GST tag
<b><u>Amino acid sequence of expressed protein</u></b>	<p>MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMTVEQNVLQOSAAQKHQQT <b>FLNQLREITGINDTQILOQALKDSNGNLELAVAFLTAKNAKTPQOETTY</b> <b>YQTALPGNDRIISVGSQADTNVIDLTGDDKDDLQRAIALSLAESNRAFRE</b> <b>TGITDEEQAISRVLEASIAENKACLKRTPTVWRDSRNPYDRKRQDKAPV</b> <b>GLKNVGNTCWFSAVIQSLFNLLEFRRLVLNYPKPSNAQDLPRNQKEHRNL</b> <b>PFMRELRYLFALLVGTKRKYVDPSRAVEILKDAFKSNDSSQQQDVSEFTHK</b> <b>LLDWLEDAFQMKAEETDEEKPKNPMVELFYGRFLAVGVLEGKKFENTEM</b> <b>FGQYPLQVNGFKDLHECLEAAMIEGEIESLHSENSGKSGQEHWFTELPPV</b> <b>LTFELSRFEFNQALGRPEKIHNKLEFPQVLYLDRYMHRNREITRIKREEI</b> <b>KRLKDYLTVLQORLERYLSYSGPKRFPPLVDVLOYALEFASSKPVCTSPV</b> <b>DDIDASSPPSGSIPSQTLPTSTTEQQGALSSELPTSTSPSSVAAISSRSVIH</b> <b>KPFTQSRIPDLPMHPAPRHITTEELSVLESCLHRWRTEIENDTRDLQES</b> <b>ISRIHRTIELMYSKSMIQVPYRLHAVLVHEGQANAGHYWAYIFDHRESR</b> <b>WMKYNDIAVTKSSWEELVRDSFGGYRNASAYCLMYINDKAQFLIQEEFNK</b> <b>ETGQPLVGIETLPPDLRDFVEEDNORFEKELEEWDAQLAQKALQEKLLAS</b> <b>QKLRESETSVTTAQAAGDPEYLEQPSRSDFSKHLKEETIQIITKASHEHE</b> <b>DKSPETVLQSAIKLEYARLVKLAQEDTPPETDYRLHHVVVYFIQNQAPKK</b> <b>IEKTLLEQFGDRNLSFDERCHNIMKVAQAKLEMIPPEVNLEEYEEWHQ</b> <b>DYRKFRETTMYLIIGLENFORESYIDSLFLICAYQNNKELLSKGLYRGH</b> <b>DEELISHYRRECLLKLNEQAAELFESGEDREVNGLIIMNEFIVPFLPLL</b> <b>LVDMEEKDILAVEDMRNRWCSYLGQEMEPHLOEKLTDFLPKLLDCSMEI</b> <b>KSFHEPKLPSYSTHELCECFARIMLSLSRTPADGR</b></p>
<b><u>Native sequence</u></b>	in bold
<b><u>Protease cleavage</u></b>	Precision site underlined
<b><u>Cloning sites</u></b>	BamHI/SalI

**DNA sequence of insert**

ATGACCGTGGAGCAGAACGTGCTGCAGCAGAGCGCGGCGCAGAAGCACCA  
GCAGACGTTTTTGAATCAACTGAGAGAAATTACGGGGATTAATGACACCC  
AGATACTACAGCAAGCCTTGAAGGATAGTAATGGAACTTGAATTAGCA  
GTGGCTTTCCTTACTGCGAAGAATGCTAAGACCCCTCAGCAGGAGGAGAC  
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GATCTTCAGAGAGCAATTGCCTTGAGTTTGGCCGAATCAAACAGGGCATT  
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CCTGCTGATGGAAGATAA