

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of GST-UCHL5**

<b><u>Enzyme description:-</u></b>	GST-UCHL5
<b><u>Clone number:-</u></b>	DU12810
<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	64260 Da
Average Mass	64300 Da
[cysteines reduced, methionines have not been oxidised]	

**Theoretical pI:-** 5.63

**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-UCHL5**

**Protein** GST-UCHL5

**Synonyms**

**Clone Number** DU12810

**Species** Human

**Accession Number** Protein: NP\_001186190.1 DNA: AAH15521.1

**Tags** N-terminal GST tag

**Amino acid sequence of expressed protein** MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFOGPLGSMTGNAGEWCLMESDPGVFT**ELIKFGCRGAQVEE**IWSLEPENFEKLKPVHGLIFLFWQPGEEPAGSVV**QDSRLDTIFFAKQVINNACATQAI**VSVLLNCTH**QDVHLGETLSEFKEFSQSFDAAMKGLALSNSDVIRQVHNSFARQOMFEFDTKTSAKEEDAFHFVSYPVNGRLYELDGLREGPIDLGACNQDDWISAVRPVIEKRIQKYSEGEIRFNLM**AI VSDRKMIYE**QKIAELQRQLAEEPMDTDQ**GNSM**LSAIQSEVAKNQMLIEEEVQKLKRYKIENIRRKHNYLPFIMELLKTLAEHQQLIPLVEKAKEKQNAKKAQETK**

**Native sequence** in bold

**Protease cleavage** Precision site underlined

**Cloning sites** BamH1 / Not1

**DNA sequence of  
insert**

GGATCCATGACGGGCAATGCCGGGGAGTGGTGCCTCATGGAAAGCGACCC  
CGGGGTCTTCACCGAGCTCATTAAGGATTCGGTTGCCGAGGAGCCCAAG  
TAGAAGAAATATGGAGTTTAGAGCCTGAGAATTTTGAAAAATTAAAGCCA  
GTTTCATGGGTAAATTTTTCTTTTCAAGTGGCAGCCAGGAGAAGAACCAGC  
AGGCTCTGTGGTTCAGGACTCCCGACTTGACACGATATTTTTTGCTAAGC  
AGGTAATTAATAATGCTTGTGCTACTCAAGCCATAGTGAGTGTGTTACTG  
AACTGTACCCACCAGGATGTCCATTTAGGCGAGACATTATCAGAGTTTAA  
AGAATTTTCACAAAGTTTTGATGCAGCTATGAAAGGCTTGGCACTGAGCA  
ATTCAGATGTGATTCGACAAGTACACAACAGTTTCGCCAGACAGCAAATG  
TTTGAATTTGATACGAAGACATCAGCAAAAAGAAGAAGATGCTTTTCACTT  
TGTCAGTTATGTTCCCTGTTAATGGGAGACTGTATGAATTAGATGGATTAA  
GAGAAGGACCGATTGATTTAGGTGCATGCAATCAAGATGATTGGATCAGT  
GCAGTAAGGCCCTGTCATAGAAAAAAGGATACAAAAGTACAGTGAAGGTGA  
AATTCGATTTAATTTAATGGCCATTGTGTCTGACAGAAAAATGATATATG  
AGCAGAAGATAGCAGAGTTACAAAGACAAC TTGCAGAGGAACCCATGGAT  
ACAGATCAAGGTAATAGTATGTTAAGTGCTATTCAGTCAGAAGTTGCCAA  
AAATCAGATGCTTATTGAAGAAGAAGTACAGAAATTA AAAAGATACAAGA  
TTGAGAATATCAGAAGGAAGCATAATTATCTGCCTTTCATTATGGAATTG  
TTAAAGACTTTAGCAGAACACCAGCAGTTAATACCACTAGTAGAAAAGGC  
AAAAGAAAAACAGAACGCAAAGAAAGCTCAGGAAACCAAATGAGCGGCCGC