

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UCHL3

<u>Enzyme description:-</u>	GST-UCHL3
<u>Clone number:-</u>	DU21015
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	N-terminal GST tag
<u>Purification method:-</u>	GSH sepharose
<u>Expression level:-</u>	0.3 mg/L

Calculated molecular mass:-

Monoisotopic	53951 Da
Average Mass	53984 Da
[cysteines reduced, methionines have not been oxidised]	

<u>Theoretical pI:-</u>	5.19
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<u>Purity:-</u>	80%
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Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

<u>Storage temperature:-</u>	-80°C
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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-UCHL3

<u>Protein</u>	GST-UCHL3
<u>Synonyms</u>	
<u>Clone Number</u>	DU21015
<u>Species</u>	Human
<u>Accession Number</u>	Protein: P15374 DNA: AAH18125.1
<u>Tags</u>	N-terminal GST
<u>Amino acid sequence of expressed protein</u>	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFP NLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAETSMLEGAVLDIRYGV SRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDAL DVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLOGWQATFGG GDHPPKSDLEVLFOGPLGSPGIPGSTRAAAMEGQRWLPLEANPEVTNQFLKQL GLHPNWQFVDVYGMDPELLSMVPRPVCAVLLLFPITEKYEVFRTEEEEKIKSQ GQDVTSSVYFMQTISNACGTIGLIHAIANNKDKMHFESGSTLKKFLEESVSM SPEERARYLENYDAIRVTHETSAHEGQTEAPSIDEKVDLHFIALVHVDGHLYE LDGRKPFPINHGETSDETLLEDAIEVCKKFMERDPDELRFNAIALSAA
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	NotI
<u>DNA sequence of insert</u>	GCGGCCGCGATGGAGGGTCAACGCTGGCTGCCGCTGGAGGCCAATCCCGA GGTCACCAACCAGTTTCTTAAACAATTAGGTCTACATCCTAACTGGCAAT TCGTTGATGTATATGGAATGGATCCTGAACTCCTTAGCATGGTACCAAGA CCAGTCTGTGCAGTCTTACTTCTCTTTCTATTACAGAAAAGTATGAAGT ATTCAGAACAGAAAGAGGAAGAAAAAATAAAATCTCAGGGACAAGATGTTA CATCATCAGTATATTTTCATGAAGCAAACAATCAGCAATGCCTGTGGAACA ATTGGACTGATTCATGCTATTGCAAACAATAAAGACAAGATGCACTTTGA ATCTGGATCAACCTTGAAAAAATTCCTGGAGGAATCTGTGTCAATGAGCC CTGAAGAACGAGCCAGATACCTGGAGAACTATGATGCCATCCGAGTTACT CATGAGACCAGTGCCCATGAAGGTCAGACTGAGGCACCAAGTATAGATGA GAAAGTAGATCTTCATTTTATTGCATTAGTTCATGTAGATGGGCATCTCT ATGAATTAGATGGGCGGAAGCCATTTCCAATTAACCATGGTGAAACTAGT GATGAACTTTATTAGAGGATGCCATAGAAGTTTGCAAGAAGTTTATGGA GCGCGACCCTGATGAACTAAGATTTAATGCGATTGCTCTTCTGCAGCAT AGGCGGCCGC