

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-USP14

<u>Enzyme description:-</u>	His-USP14
<u>Clone number:-</u>	DU14352
<u>Source:-</u>	BL21 Recombinant
<u>Tag:-</u>	His
<u>Purification method:-</u>	Ni ⁺⁺ -Sephrose
<u>Expression level:-</u>	3 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	58476 Da
Average Mass [cysteines reduced, methionines have not been oxidised]	58512 Da
<u>Theoretical pI:-</u>	5.57
<u>Purity:-</u>	80%
<u>Enzyme storage buffer:-</u>	
	50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT
<u>Storage temperature:-</u>	-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-USP14

<u>Protein</u>	His-USP14
<u>Synonyms</u>	UBP14, TGT
<u>Clone Number</u>	DU14352
<u>Species</u>	Human
<u>Accession Number</u>	Protein: P54578 DNA: AAH03556.1
<u>Tags</u>	N-terminal His ₆ tag
<u>Amino acid sequence of expressed protein</u>	MGSSHHHHHSSGLEVLFGPGSMP <u>LYSVTVKWKGEKFE</u> GVELNTDEPPM VFKAQLFALTGVQPARQKVMVKG <u>GT</u> LKDDDWGNIKIKNGMTLLMMGSADA LPEEPSAKTVFVEDMTEEQLASAME <u>LPCGLTNLGN</u> TCYMNATVQCI <u>RSVP</u> ELKDALKRYAGALRASGEMASAQY <u>ITAALRDLFDSMDKTSSS</u> IPPIILLO FLHMAFPQFAEKGEQGOYLQODANE <u>CWIQMMRVLOQKLEA</u> IEDDSVKETD SSSASAATPSKKKSLIDQFFGVEFET <u>TMKCTESE</u> EEEEVTGKQENQLQLSC FINQEVKYLFTGLKLRLOEEITKQSP <u>TLQRNALYIKSSKISRLPAYLTIQ</u> MVRFFYKEKESVNAKVLKDVKFLMLD <u>MYELCTPELQEKMVSFRSKFKDL</u> EDKKNQOPNTSDKKSSPOKEVKYEP <u>FSFADDIGSNNCGYYDLQAVLTHQ</u> GRSSSSGHYVSWVRRKQDEWIKFDD <u>DKVSI</u> VTPELILRLSGGGDWHIAYV LLYGPRRVEIMEESEQ
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	BamH1 / Not1

**DNA sequence of
insert**

GGATCCATGCCGCTCTACTCCGTTACTGTAAAATGGGGAAAGGAGAAATT
TGAAGGTGTAGAATTGAATACAGATGAACCTCCAATGGTATTC AAGGCTC
AGCTGTTTGCCTTGACTGGAGTCCAGCCTGCCAGACAGAAAAGTTATGGTG
AAAGGAGGAACGCTAAAGGATGATGATTGGGGAAACATCAAAAATAAAAA
TGGAATGACTCTACTAATGATGGGGTCAGCAGATGCTCTTCCAGAAGAAC
CCTCAGCCAAAACGTCTTTCGTAGAAGACATGACAGAAGAACAGTTAGCA
TCTGCTATGGAGTTACCATGTGGATTGACAAAACCTTGGAACACTTGTTA
CATGAATGCCACAGTTCAGTGTATTTCGTTCTGTGCCTGAACTCAAAGATG
CCCTTAAAAGGTATGCAGGTGCCTTGAGAGCTTCAGGGGAAATGGCTTCA
GCGCAGTATATTACTGCAGCCCTTAGAGATTTGTTTGATTCCATGGATAA
AACTTCTTCCAGTATTCCACCTATTATTCTACTGCAGTTTTTGCACATGG
CTTCCCACAGTTTGCCGAGAAAAGGTGAACAAGGACAGTATCTTCAACAG
GATGCTAATGAATGTTGGATACAAATGATGCGAGTATTGCAACAGAAATT
GGAAGCAATAGAGGATGATTCTGTTAAAGAGACAGACTCCTCATCTGCAT
CGGCAGCGACACCTTCTAAAAAGAAAAGTTAATCGATCAGTTCTTCGGT
GTTGAGTTTGAAACTACCATGAAATGTACAGAATCTGAAGAAGAAGAAGT
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AAGTCAAGTATCTTTTTACAGGACTTAAATTGCGACTTCAGGAAGAAATC
ACCAAACAGTCTCCAACGTTGCAAAGAAATGCCTTGTATATCAAATCTTC
CAAGATCAGCCGGCTGCCTGCTTACTTGACCATTTCAGATGGTTCGATTTT
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CTCGCAGAGTTGAAATAATGGAAGAGGAAAAGTGAACAGTAAGCGGCCGC