

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of His-USP10**

<b><u>Enzyme description:-</u></b>	His-USP10
<b><u>Clone number:-</u></b>	DU15647
<b><u>Source:-</u></b>	Sf21 Recombinant
<b><u>Tag:-</u></b>	N-terminal His
<b><u>Purification method:-</u></b>	Ni <sup>++</sup> -Sepharose
<b><u>Expression level:-</u></b>	5 mg/L

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

Monoisotopic	91912 Da
Average Mass	91967 Da
[cysteines reduced, methionines have not been oxidised]	

**Theoretical pI:-** 5.39

**Purity:-** 80%

#### **Enzyme storage buffer:-**

50mM 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**

**His-USP10**

<b><u>Protein</u></b>	His-USP10
<b><u>Synonyms</u></b>	
<b><u>Clone Number</u></b>	DU15647
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: Q14694 DNA: AAH00263.1
<b><u>Tags</u></b>	N-terminal His <sub>6</sub> tag
<b>Amino acid sequence of expressed protein</b>	<b>MSYYHHHHHDYDIPTTENLYFQGAMGSGIQRPTSTSSLVAAAMALHSPQYIF GDFSPDEFNQFFVTPRSSVELPPYSGTVLCGTQAVDKLPDGOEYQRIEFGVDE VIEPSDTLPRTPSYSISSTLNPQAPEFILGCTASKITPDGITKEASYGSIDCQ YPGSALALDGSSNVEAEVLENDGVSGGLGQERKKKKRPPGYYSYKDGDD SISTEALVNGHANSVAVNSVSAEDAEMGDMPPPLTPRTCNSPQNSTDSVSDI VPDSPFPGALGSDTRTAGOPEGGPGADFGQSCFPAEAGRDTLSRTAGAQPCVG TDTTENLGVANGQILESSGEGTATNGVELHTTESIDLDPKPEASPPADGTG SASGTLPVSQPKSWASLFHDSKPSSSSPVAVVETKYSPPAISPLVSEKQVEVK EGLVPVSEDPVAIKIAELLENVTLIHKPVSLQPRGLINKGNWCYINATLQALV ACPPMYHLMKFIPLYSKVQRPCSTSTMIDSFVRLMNEFTNMPVPPKPRQALGD KIVRDIRPAAFEPTYIYRLLTVNKSSLSEKGRQEDAEYLGFI LNGLHEEML NLKLLSPSNEKLTISNGPKNHSVNEEQEEQEGSEDEWEQVGNKTSVTR QADFVQTPITGIFGGHIRSVVYQSSKESATLQPFFTLQLDIQSDKIRTVQDA LESLVARESVOGYTTTKQVEISRRTLEKLPVVLVHLKRFVYEKTGGCQK LIKNIYVPVDLEISKELLSPGVKNKFKCHRTRFAVVYHHGNSATGGHYTT DVFQIGLNGWLRIDDQTVKVINQYQVVKPTAERTAYLLYRRVDLL</b>
<b>Native sequence</b>	in bold
<b>Protease cleavage</b>	TEV cleavage site underlined
<b>Cloning sites</b>	NotI / NotI

**DNA sequence of  
insert**

CGGCGCGGATGGCCCTCCACAGCCCGCAGTATATTTTTGGAGATTTTAGCCC  
TGATGAATTC AATCAATTC TTTGTGACTCCTCGATCTTCAGTTGAGCTTCCTC  
CATACAGTGG AACAGTTCTGTGTGGCACACAGGCTGTGGATAAACTACCTGAT  
GGACAAGAATATCAGAGAATTGAGTTTGGTGTGCGATGAAGTCATTGAACCCAG  
TGACACTTTGCCGAGAACCCCGAGCTACAGTATTTCAAGCACACTGAACCCCTC  
AGGCCCTGAATTTATTCTCGGTTGTACAGCTTCCAAAATAACCCCTGATGGT  
ATCACTAAAGAAGCAAGCTATGGCTCCATCGACTGCCAGTACCCAGGCTCTGC  
CCTCGCTTTGGATGGAAGTTCTAATGTGGAGGCGGAAGTTTTGGAAAATGATG  
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TTTCCCCGGAGCACTCGGCAGTGACACCAGGACTGCAGGGCAGCCAGAGGGGG  
GCCCCGGGGCTGATTTTGGTCAGTCTTCCCTGCAGAGGCTGGCAGAGAC  
ACCCTGTCAAGGACAGCTGGGGCTCAGCCCTGCGTTGGTACCGATACTACTGA  
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TCTCTCACCAAGTAATGAAAAACTTACGATTTCCAAACGGCCCCAAAACCACT  
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TCAGACTCCAATCACCGGCATTTTTTGGTGGACACATCAGGTCTGTGGTTTACC  
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GATATCCAGTCAGACAAGATACGCACAGTCCAGGATGCACTGGAGAGCTTGGT  
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TAAGTCGAAGAGTGAAGTCTGGAAAACTCCCTCCTGTCTCGTGTGCACCTG  
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TGAATATCCTGTGGACTTGGAAATTAGTAAAGAAGTCTTTCTCCAGGGGTTA  
AAAATAAGAATTTTAAATGCCACCGAACCCTATCGGCTCTTTGCAGTGGTCTAC  
CATCACGGCAACAGTGCAGCGGGCGCCATTACACTACAGACGTCTTCCAGAT  
CGGTCTGAATGGCTGGCTGCGCATCGATGACCAGACAGTCAAGGTGATCAACC  
AGTACCAGGTGGTGAACCAACTGCTGAACGCACAGCCTACCTCCTGTATTAC  
CGCCGAGTGGACCTGCTGTAAGCGGCCGC