

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-USP5

<u>Enzyme description:-</u>	USP5
<u>Clone number:-</u>	DU15641
<u>Source:-</u>	E. coli Recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -Sephrose
<u>Expression level:-</u>	5 mg/L

Calculated molecular mass:-

Monoisotopic	98820 Da
Average Mass	98880 Da
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 4.96

Purity: 90%

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

His-USP5

<u>Protein</u>	USP5
<u>Synonyms</u>	Isopeptidase T
<u>Clone Number</u>	DU15641
<u>Species</u>	Human
<u>Accession Number</u>	Protein: P45974 DNA: U47927.1
<u>Tags</u>	N-terminal His ₆ tag
<u>Amino acid sequence of the expressed protein</u>	MGSSHHHHHSSGLEVLFOGPGSPNSPLGSMALSEEALLSVLPTIRVPK AGDRVHKDECAFSFDTPESEGGLYICMNTFLGFGKQYVERHFNKTGORVY LHLRRTRRPKEEDPATGTGDPPrKKPTRLAIGVEGGFDLSEEKfelDEDV KIVILPDYLEIARDGLGGLPDIVRDRVTSAVEALLSADSASRKQEVQAWD GEVRQVSKHAFSLKQLDNPARIPPCGWKCSKCDMRENLWLNLTGDSILCG RRYFDGSGGNNAVEHYRETGYPLAVKLGITIPDGADVYSYDEDDMVLDP SLAEHLSHFGIDMLKMOKTDKTMTELEIDMNQRIGEWELIQESGVPLKPL FGPGYTGIRNLGNSCYLNSVVQVLFsIPDFORKYVDKLEKIFQNAPTDPT QDFSTQVAKLGHGLLSGEYSKVPESGDGERVPEQKEVQDGIAPRMFKAL IGKGHPEFSTNRQDAQEFFLHLINMVERNCRSSENPNVFRFLVEEKIK CLATEKVKYTORVDYIMQLPVPMDAALNKEELLEYEKKRQAEEEKMALP ELVRAQVPFSSCLEAYGAPEQVDDFWSTALQAKSVAVKTRFASFDPDYL IQIKKFTFGLDWVPKLDVSIEMPEELDISQLRGTGLQPGEEELPDIAPP LVTPDEPKGSLGFYGNEDDSFCSPHFSSPTSPMLDESVIQLVEMGFPM DACRKAVYYTGNSGAEAAAMNWVMHMDPDFANPLILPGSSGPGSTSAAA DPPPEDCVTTIVSMGFSRDQALKALRATNNSLERAVDWIFSHIDDLDAEA AMDISEGRSAADSISESVPVGPkVRDGPgKYQLFAFISHMGTSTMCgHYV CHIKKEGRWVIYNDQKVCASEKPPKDLGYIYFYQrVAS
<u>Native sequence</u>	in bold
<u>Protease cleavage</u>	Precision site underlined
<u>Cloning sites</u>	BamH1 / Not1

**DNA sequence of
insert**

GGATCCATGGCGGAGCTGAGTGAGGAGGCGCTGCTGTCAGTATTACCGACGA
TCCGGGTCCCTAAGGCTGGAGACCGGGTCCACAAAGACGAGTGCGCCTTCTC
CTTCGACACGCCGGAGTCTGAGGGGGCCTCTACATCTGTATGAACACGTTT
CTGGGCTTTGGGAAACAGTATGTGGAGAGACATTTCAATAAGACCGGCCAGC
GAGTCTACTTGCACCTCCGGCGGACCCGGCGCCGAAAGAGGAGGACCCTGC
TACAGGCACTGGAGACCCACCCCGGAAGAAGCCACGCGGCTGGCTATTGGT
GTTGAAGGCGGATTTGACCTTAGCGAGGAGAAGTTTGAATTAGACGAGGATG
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