

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of His-USP8

<u>Enzyme description:-</u>	His-USP8
<u>Clone number:-</u>	DU15645
<u>Source:-</u>	Sf21 Recombinant
<u>Tag:-</u>	N-terminal His ₆ -tag
<u>Purification method:-</u>	Ni ⁺⁺ -Sephadex
<u>Expression level:-</u>	5 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	130711 Da
Average Mass	130789 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	8.91
<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-USP8

Protein USP8 with N-terminal His-tag
Synonyms UBPY
Clone Number DU15645
Species Human
Accession Number Protein: P40818 DNA: NM_005154.3
Tags N-terminal His₆-tag

Amino acid sequence of expressed protein MSYYHHHHHDYDIPTTENLYFQAGMGSMPAVASVPKELYLSSSLKDLNKKTE
VKPEKISTKSYVHSALKIFKTAEECRDRDEERAYVLYMKYVTVYNLIKRRPD
FKQQDYFHSILGPGNIKKAVEEAERLSESLKLRYEAEVVRKKLEEKDRQEEA
QRLQQRQETGREDGGTLAKGSLENVLDSDKDKTQKSNGEKNEKCETKEKGAIT
AKELYTMMDKNI SLIIMDARRMQDYQDSCILHSLSVPPEAISPQVGTASWIEA
HLPDDSKDTWKKRGNVEYVLLDFSSAKDLQIGTTLRSLKDALFKWESKTVL
RNEPLVLEGGYENWLLCYPQYTTNAKVTPPPRRQNEEVSISLDFTYPSLEESI
PSKPAAQTTPASIEVDENIELISGQNERMGPLNISTPVEPVAASKSDVSPIIQ
PVPSIKNVPOIDRTKKPAVKLPEEHRIKSESTNHEQQSPQSGKVI PDRSTKPV
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RSYSSPDITQAIQEEKRPVTPVTVNRENKPTCYPKAEISRLSASQIRNLNP
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NNRFYCSHCRRARDSLKKIEIWKLPPVLLVHLKRFSDGRWKQKLQTSVDFPL
ENLDLSQYVIGPKNNLKKYNLFSVSNHYGGLDGGHYTAYCKNAARQRWFKFDD
HEVSDISVSSVKSSAAYILFYTSLGPRVTDVAT

Native sequence in bold
Protease cleavage TEV site underlined
Cloning sites BamH1 / Not1

DNA sequence of insert

GGATCCATGCCTGCTGTGGCTTCAGTTCCTAAAGAACTCTACCTCAGTTCCTC
ACTAAAAGACCTTAATAAGAAGACAGAAGTTAAACCAGAGAAAATAAGCACTA
AGAGTTATGTGCACAGTGCCCTGAAGATCTTTAAGACAGCAGAAGAATGCAGA
TTAGATCGTGATGAGGAAAGGGCCTATGTACTATATATGAAATACGTGACTGT
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