

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Halo-Ubiquitin (pSer65)

<u>Enzyme description:-</u>	Ubiquitin pSer65 (1-76) full length mature
<u>Clone number:-</u>	DU24950
<u>Source:-</u>	human recombinant
<u>Tag:-</u>	His-Halo
<u>Purification method:-</u>	Ni-agarose; phosphorylated by PINK1
<u>Expression system:-</u>	E.coli
<u>Calculated molecular mass:-</u>	
Monoisotopic	44286 Da
Average Mass	44313 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	< 5.31
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50 mM Tris pH 7.5, 150mM NaCl, 1mM DTT	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	

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Clone Data Sheet

Ubiquitin (pSer65)

<u>Protein</u>	Ubiquitin (pSer65) (1-76) full length mature
<u>Synonyms</u>	
<u>Clone Number</u>	DU24950
<u>Species</u>	Human
<u>Accession Number</u>	P62987
<u>Tags</u>	N-terminal His-Halo
Aminoacid sequence of the expressed protein	MHHHHHMAEIGTGFPFDPHYVEVLGERMHYVDVGP RDGTPVLF L HGNPT SSYVWRNIIPHVAPTHRCIAPDLIGMGKSDKPD L GYFFDDHVRFM DAFIE ALGLEEVVLVIHDWGSALGFHWAKRNP ERVKGIAFMEFIRPIPTWDEWPE FARETFQAFRTT DVGRKLIIDQNVFIEGTLPMGVVRPLTEVEMDHYREPF LNPVDREPLWRFPNELPIAGEPANIVALVEEYMDWLHQSPVPKLLFWGTP GVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDLIGSEIARWLSTL EISGENLYFOGGSAGM QIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEG IPPDQQRLIFAGKQLEDGRTLSDYNIQKEpSTLHLVLRRLGG
Native sequence	mature full length ubiquitin 1-76 in bold.
Protease cleavage	TEV-protease site underlined
Cloning sites	BamH1 / Not1

**DNA sequence
of insert**

ATGCATCACCATCACCATCACATGGCAGAAATCGGTACTGGCTTTCCATT
CGACCCCATTTATGTGGAAGTCCTGGGCGAGCGCATGCACTACGTTCGATG
TTGGTCCGCGGATGGCACCCCTGTGCTGTTCCCTGCACGGTAACCCGACC
TCCTCCTACGTGTGGCGCAACATCATCCCGCATGTTGCACCGACCCATCG
CTGCATTGCTCCAGACCTGATCGGTATGGGCAAATCCGACAAACCAGACC
TGGGTTATTTCTTCGACGACCACGTCCGCTTCATGGATGCCTTCATCGAA
GCCCTGGGTCTGGAAGAGGTCGTCCTGGTCATTCACGACTGGGGCTCCGC
TCTGGGTTTCCACTGGGCCAAGCGCAATCCAGAGCGCGTCAAAGGTATTG
CATTTATGGAGTTCATCCGCCCTATCCCGACCTGGGACGAATGGCCAGAA
TTTGCCCGGAGACCTTCCAGGCCTTCCGCACCACCGACGTCGGCCGCAA
GCTGATCATCGATCAGAACGTTTTTATCGAGGGTACGCTGCCGATGGGTG
TCGTCCGCCCGCTGACTGAAGTCGAGATGGACCATTACCGCGAGCCGTT
CTGAATCCTGTTGACCGCGAGCCACTGTGGCGCTTCCCAAACGAGCTGCC
AATCGCCGGTGAGCCAGCGAACATCGTCGCGCTGGTCGAAGAATACATGG
ACTGGCTGCACCAGTCCCCTGTCCCGAAGCTGCTGTTCTGGGGCACCCCA
GGCGTTCTGATCCACCGGCCGAAGCCGCTCGCCTGGCCAAAAGCCTGCC
TAACTGCAAGGCTGTGGACATCGGCCCGGGTCTGAATCTGCTGCAAGAAG
ACAACCCGGACCTGATCGGCAGCGAGATCGCGCGCTGGCTGTCGACGCTC
GAGATTTCCGGCGAAAACCTGTATTTTCAGGGCGGATCCGCCGGCATGCA
GATCTTCGTGAAGACCCTGACTGGTAAGACCATCACTCTCGAAGTGGAGC
CGAGTGACACCATTGAGAATGTCAAGGCAAAGATCCAAGACAAGGAAGGC
ATCCCTCCTGACCAGCAGAGGTTGATCTTTGCTGGGAAACAGCTGGAAGA
TGGACGCACCCTGTCTGACTACAACATCCAGAAAGAGTCCACCCTGCACC
TGGTCCTCCGTCTCAGAGGCGGCTGATAAGCGGCCGC