

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin 80-465 [R163A]

<u>Enzyme description:-</u>	Parkin 80-465 [R163A]
<u>Clone number:-</u>	DU43844
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	cleaved from N-terminal His-SUMO1 tag
<u>Purification method:-</u>	Ni ⁺⁺ -Sephrose, SENP-treatment, Ni ⁺⁺ -depletion, SEC
<u>Expression level:-</u>	1mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	42406Da
Average Mass	42433Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.92
<u>Purity:-</u>	90%
<u>Enzyme storage buffer:-</u>	
50mM HEPES pH 8.2, 150mM NaCl, 20% glycerol, 1mM TCEP	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	

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Clone Data Sheet

Parkin 80-465 [R163A]

<u>Protein</u>	Parkin 80-465 [R163A]
<u>Synonyms</u>	PARK2, PRKN2
<u>Clone Number</u>	DU43844
<u>Species</u>	Human
<u>Accession Number</u>	Protein: O60260 Gene: NM_004562.2
<u>Tags</u>	N-terminal His ₆ -SUMO1-tag (cleaved)
Aminoacid sequence of the expressed protein	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTTH LKLLKESYQORQGVPMNSLRFLFEGQRIADNHTPKELGMEEDVIEVYQE QTGGMNATGGDDPRNAAGGCERE PQSLTRVDLSSSVLPGDSVGLAVILHT DSRKDSPPAGSPAGRSIYNSFYVYCKGPCQRVQPGKLAVQCSTCRQATLT LTQGPSCWDDVLI PNRMSGEC QSPHCPGTSAEFFFKCGAHPTSDKETSVA LHLIATNSRNITCITCTDVRSPVLVFCNSRHVICLDCFHLYCVTRLNDR QFVHDPQLGYSLPCVAGCPNSLIKELHHFRILGEEQYNRYQQYGAEECVL QMGGVLCPRPGCCAGLLPEPDQRKVTCEGGNGLGCGFAFCRECKEAYHEG ECSAVFEASGTTTQAYRVDERAAEQARWEAASKETIKKTTKPCPRCHVPV EKNGGCMHMKCPQPQCRLEWCWNCGEWNRVCMGDHWFVDV
Native sequence	in bold
Protease cleavage	SEN1 treatment after GG of SUMO1
Cloning sites	
<u>DNA sequence of cassette</u>	CCATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATAATAACTCAAAGT CATTGGACAGGATAGCAGTGAGATTCACCTCAAAGTGAAAATGACAACAC ATCTCAAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTTCATG AACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATA TCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGG AACAAACGGGGGaatgaatgcaactggagggcagacccccagaaacgcg gcgggaggctgtgagcgggagccccagagcttgactcgggtggacctcag cagctcagtcctcccaggagactctgtggggctggctgtcattctgcaca ctgacagcaggaaggactcaccaccagctggaagtccagcaggttagatca atctacaacagcttttatgtgtattgcaaaggccccctgtcaaagagtgca gccgggaaaactcGCggtacagtgagcacctgcagggcaggcaacgctca ccttgaccagggtccatcttgctgggatgatgttttaattccaaaccgg atgagtggatgaatgccaatccccacactgccctgggactagtgcagaatt ttcttttaaatgtggagcacacccccacctctgacaaggaacatcagtag ctttgcacctgatcgcaacaatagtcggaacatcacttgacattacgtgc acagacgtcaggagccccgtcctgggtttccagtgcaactcccgccacgt gatttgcttagactgtttccacttatactgtgtgacaagactcaatgatc ggcagtttggtcacgacctcaacttggctactccctgccttgtgtggct ggctgtcccaactccttgattaaagagctccatcacttcaggattctggg agaagagcagtacaaccggtaccagcagtatggtgcagaggagtgtgtcc tgcagatggggggcgtgttatgccccgccctggctgtggagcggggctg ctgccggagcctgaccagaggaaagtcacctgcgaagggggcaatggcct gggctgtggggtttgccttctgccgggaatgtaaagaagcgtaccatgaag

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