

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin T415N

<u>Enzyme description:-</u>	Parkin 1-465 T415N
<u>Clone number:-</u>	DU39786
<u>Source:-</u>	BL21 recombinant
<u>Tag:-</u>	cleaved from N-terminal His-SUMO1 tag
<u>Purification method:-</u>	Ni ⁺⁺ -Sephareose, SENP-treatment, Ni ⁺⁺ -depletion, SEC
<u>Expression level:-</u>	0.2mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	51619 Da
Average Mass	51652 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.21
<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 T415N

<u>Protein</u>	Parkin 1-465 T415N
<u>Synonyms</u>	PARK2, PRKN2
<u>Clone Number</u>	DU39786
<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	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTTHLK KLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEDVIEVYQEQTGG MIVFVRFNSSHGFVEVDSDTSI FQLKEVVAKRQGV PADQLRVI FAGKELRN DWTVQNCDDLQQSIVHIVQRPWRKQEMNATGGDDPRNAAGGCEREPOSLTR VDLSSSVLPGDSVGLAVILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQR VQPGKLRVQCSTCRQATLTLTQGPSCWDDVLI PNRMSGECQSPHCPGTSAEF FFKCGAHTSDKETSVALHLIATNSRNITCITCTDVRSPVLVFCNSRHVIC LDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNSLIKELHFRILGEEQY NRYQQYGAEECVLQMGVLCPRPGCGAGLLPEPDQRKVTCEGGNGLGCGFAF CRECKEAYHEGECSAVFEASGTTTQAYRVD ERAAEQARWEAASKETIKKTNK PCPRCHVPVEKNGGCMHMKCPQPQCRLEWCWNCGEWNRVCMGDHWFVDV
Native sequence	in bold
Protease cleavage	SEN1 treatment after GG of SUMO1
Cloning sites	BamH1 / Not 1 (destroyed)
<u>DNA sequence of the insert</u>	atgatagtgtttgtcaggttcaactccagccatggtttcccagtgagggtcg attctgacaccagcatcttccagctcaaggaggtggttgctaagcgacaggg ggttccggctgaccagttgctgtgattttcgcagggaaaggagctgaggaat gactggactgtgcagaattgtgacctggatcagcagagcattgttcacattg tgcagagaccgtggagaaaagggtcaagaaatgaatgcaactggaggcgacga ccccagaaacgcggcgggaggctgtgagcgggagccccagagcttgactcgg gtggacctcagcagctcagtcctcccaggagactctgtggggctggctgtca ttctgcacactgacagcaggaaggactcaccaccagctggaagtccagcagg tagatcaatctacaacagcttttatgtgtattgcaaaggccctgtcaaaga gtgcagccgggaaaactcaggggtacagtgacagcactgcaggcaggcaacgc tcaccttgaccaggggtccatcttgcctgggatgatgttttaattccaaaccg gatgagtggatgaatgccaatccccacactgccctgggactagtgcagaattt ttctttaaatgtggagcacaccccacctctgacaaggaaacaTcagtagctt tgcacctgatcgcaacaaatagtcggaacatcacttgcaattacgtgcacaga cgtcaggagccccgtcctgggtttccagtgcaactcccgccacgtgatttgc ttagactgtttccacttatactgtgtgacaagactcaatgatcggcagtttg ttcacgacctcaacttggctactccctgccttgtgtggctggctgtcccaa ctccttgattaaagagctccatcacttcaggattctgggagaagagcagtac aaccggtaccagcagtatgggtgcagaggagtgtgtcctgcagatggggggcg tggtatgccccgcctggctgtggagcggggctgctgccggagcctgacca gaggaaagtcacctgcgaagggggcaatggcctgggctgtgggtttgccttc tgccgggaatgtaaagaagcgtaccatgaaggggagtgacagtgccgtatttg aagcctcaggaacaactactcaggcctacagagtcgatgaaagagccgccga gcaggctcgttgggaagcagcctccaagaaacatcaagaaaaccaAcaag

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