

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin S223P

<u>Enzyme description:-</u>	Parkin 1-465 (full length) S223P, T415N
<u>Clone number:-</u>	DU39126
<u>Source:-</u>	Recombinant
<u>Tag:-</u>	cleaved from N-terminal His ₆ -SUMO-1
<u>Purification method:-</u>	Ni ⁺⁺ -Sepharose, SEC
<u>Expression level:-</u>	1 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	51629 Da
Average Mass	51662 Da
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	7.21
<u>Purity:-</u>	95 %
<u>Enzyme storage buffer:-</u>	
50 mM HEPES pH 8.2, 20% glycerol, 150mM NaCl, 0.5mM TCEP, 0.03% Brij35	
<u>Storage temperature:-</u>	-80°C
<u>Assay:-</u>	

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Clone Data Sheet

Protein name Parkin

<u>Protein</u>	Parkin 1 - 465 (full length) S223P, T415N (rare variant)
<u>Synonyms</u>	PARK2, PRKN
<u>Clone Number</u>	DU39126
<u>Species</u>	Human
<u>Accession Number</u>	Protein: BAA25751
<u>Tags</u>	N-terminal His, followed by SUMO-1 to improve solubility
Aminoacid sequence of the expressed protein .	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTTH LKKLKESYCORQGVPMNSLRFLFEGQRIADNHTPKELGMEEDVIEVYQE QTGGMIVFVRFNSSHGFPVEVSDTTSIFQLKEVVAKRQGVADQLRVIFA GKELRNDWTVQNCDLDOQSI VHI VQRPWRKQEMNATGGDDPRNAAGGCE REPOSLTRVDLSSSVLPGDSVGLAVILHTDSRKDSPPAGSPAGRSIYNSF YVYCKGPCQORVQPGKLRVQCS TCRQATLTLTQGPSCWDDVLI PNRMSGEC QSPHCPGTSAEFFFKCGAHPTSDKETPVALHLIATNSRNITCITCTDVRS PVLVFOCNSRHVICLDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNS LIKELHHFRILGEEQYNRYQQYGAEECVLQMGVLCPRPGCGAGLLPEPD QRKVTCEGGNGLGCGFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDER AAEQARWEAASKETIKKTNKPCPRCHVPVEKNGGCMHMKCPQPQCRLEWC WNCGCEWNRVCMGDHWFVDV
SUMO-1 in grey, is removed during purification by SENP1	
The final product, Parkin 1- 465 in bold	
Native sequence	in bold
Protease cleavage	SENP1 protease site underlined
Cloning sites	Complex cloning, please inquire.

DNA sequence of cassette

ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCAACT
GAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAGTCAAAGTCATT
GGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACAACACATCTC
AAGAACTCAAAGAATCATACTGTCAAAGACAGGGTGTCCAATGAACTCA
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