

Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin S223P R42P

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| <u>Enzyme description:-</u> | Parkin 1-465 (full length) S223P, R42P |
| <u>Clone number:-</u> | DU39313 |
| <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> | 0.5 mg/L |
| <u>Calculated molecular mass:-</u> | |
| Monoisotopic | 51557 Da |
| Average Mass | 51590 Da |
| [cysteines reduced, methionines have not been oxidised] | |
| <u>Theoretical pI:-</u> | 7.00 |
| <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

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|---|--|
| <u>Protein</u> | Parkin 1 - 465 (full length) S223P R42P (rare variant) |
| <u>Synonyms</u> | PARK2, PRKN |
| <u>Clone Number</u> | DU39313 |
| <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 QTGGMIVFVRFNSSHGFPVEVSDTTSIFQLKEVVAKRQGVADQLPVIFA GKELRNDWTVQNCDLDOQSI VHI VQRPWRKQEMNATGGDDPRNAAGGCE REPOSLTRVDLSSSVLPGDSVGLAVILHTDSRKDSPPAGSPAGRSIYNSF YVYCKGPCQORVQPGKLRVQCS TCRQATLTLTQGPSCWDDVLI PNRMSGEC QSPHCPGTSAEFFFKCGAHPTSDKETPVALHLIATNSRNITCITCTDVRS PVLVFOCNSRHVICLDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNS LIKELHHFRILGEEQYNRYQQYGAEECVLQMGVLCPRPGCGAGLLPEPD QRKVTCEGGNGLGCGFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDER AAEQARWEAASKETIKKTTKPCPRCHVPVEKNGGCMHMKCPQPQCRLEWC WNCGCEWNRVCMGDHWF DV |
| 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
CTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATACTCCAAA
GAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGGAACAAACG
GGGGAatgatagtggttgcaggttcaactccagccatggtttcccagtg
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