Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin [R163A]

Enzyme description:- Parkin 1-465 [R163A]

Clone number:- DU43801

Source:- BL21 recombinant

Tag:- cleaved from N-terminal His-SUMO1 tag

Purification method:- Ni^{++}-Sepharose, SENP-treatment, Ni^{++}-depletion, SEC

Expression level:- 0.2mg/L

Calculated molecular mass:-

Monoisotopic	51521 Da
Average Mass	51554 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.00

Purity:- 90%

Enzyme storage buffer:-

50mM HEPES pH 8.2, 150mM NaCl, 20% glycerol, 1mM TCEP

Storage temperature:- -80ºC

Assay:-

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Clone Data Sheet

Parkin [R163A]

Protein	Parkin 1-465 [R163A]
Synonyms	PARK2, PRKN2
Clone Number	DU43801
Species	Human
Accession Number	Protein: O60260 Gene: NM_004562.2
Tags	N-terminal His_6-SUMO1-tag (cleaved)
Aminoacid sequence of the expressed protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTH LKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQE QTGG**MIVFVRFNSSHGFPVEVDSDTSIFQLKEVVAKRQGVPADQLRVIFA** **GKELRNDWTVQNCDLDQQSIVHIVQRPWRKGQEMNATGGDDPRNAAGGCE** **REPQSLTRVDLSSSVLPGDSVGLAVILHTDSRKDSPPAGSPAGRSIYNSF** **YVYCKGPCQRVQPGKLAVQCSTCRQATLTLTQGPSCWDDVLIPNRMSGEC** **QSPHCPGTSAEFFFKCGAHPTSDKETSVALHLIATNSRNITCITCTDVRS** **PVLVFQCNSRHVICLDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNS** **LIKELHHFRILGEEQYNRYQQYGAEECVLQMGGVLCPRPGCGAGLLPEPD** **QRKVTCEGGNGLGCGFAFCRECKEAYHEGECSAVFEASGTTTQAYRVDER** **AAEQARWEAASKETIKKTTKPCPRCHVPVEKNGGCMHMKCPQPQCRLEWC** **WNCGCEWNRVCMGDHWFDV**
Native sequence	in bold
Protease cleavage	SENP1 treatment after GG of SUMO1
Cloning sites	BamH1 / Not 1 (destroyed)
DNA sequence of the cassette	ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCAAC TGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAAGTCA TTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACAACACAT CTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTTCCAATGAA CTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATACTC CAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGGAA CAAACGGGGGGAatgatagtgtttgtcaggttcaactccagccatggttt cccagtggaggtcgattctgacaccagcatcttccagctcaaggaggtgg ttgctaagcgacaggggttccggctgaccagttgcgtgtgattttcgca gggaaggagctgaggaatgactggactgtgcagaattgtgacctggatca gcagagcattgttcacattgtgcagagaccgtggagaaaaggtcaagaaa tgaatgcaactggaggcgacgaccccagaaacgcggcgggaggctgtgag cgggagccccagagcttgactcgggtggacctcagcagctcagtcctccc aggagactctgtggggctggctgtcattctgcacactgacagcaggaagg actcaccaccagctggaagtccagcaggtagatcaatctacaacagcttt tatgtgtattgcaaaggcccctgtcaaagagtgcagccgggaaaactcgc ggtacagtgcagcacctgcaggcaggcaacgctcaccttgacccagggtc catcttgctgggatgatgttttaattccaaaccggatgagtggtgaatgc caatccccacactgccctgggactagtgcagaatttttctttaaatgtgg agcacaccccacctctgacaaggaaacatcagtagctttgcacctgatcg caacaaatagtcggaacatcacttgcattacgtgcacagacgtcaggagc cccgtcctggttttccagtgcaactcccgccacgtgatttgcttagactg tttccacttatactgtgtgacaagactcaatgatcggcagtttgttcacg

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