Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin [R305A]

Enzyme description:- Parkin 1-465 [R305A]

Clone number:- DU46035

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:-

Division of Signal Transduction Therapy

Clone Data Sheet

Parkin [R305A]

Protein	Parkin 1-465 [R305A]
Synonyms	PARK2, PRKN2
Clone Number	DU46035
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** **YVYCKGPCQRVQPGKLRVQCSTCRQATLTLTQGPSCWDDVLIPNRMSGEC** **QSPHCPGTSAEFFFKCGAHPTSDKETSVALHLIATNSRNITCITCTDVRS** **PVLVFQCNSRHVICLDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNS** **LIKELHHFAILGEEQYNRYQQYGAEECVLQMGGVLCPRPGCGAGLLPEPD** **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 insert	CCATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAAGT CATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACAACAC ATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTTCCAATG AACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATAC TCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGG AACAAACGGGGGGAatgatagtgtttgtcaggttcaactccagccatggt ttcccagtggaggtcgattctgacaccagcatcttccagctcaaggaggt ggttgctaagcgacaggggttccggctgaccagttgcgtgtgattttcg cagggaaggagctgaggaatgactggactgtgcagaattgtgacctggat cagcagagcattgttcacattgtgcagagaccgtggagaaaaggtcaaga aatgaatgcaactggaggcgacgaccccagaaacgcggcgggaggctgtg agcgggagccccagagcttgactcgggtggacctcagcagctcagtcctc ccaggagactctgtggggctggctgtcattctgcacactgacagcaggaa ggactcaccaccagctggaagtccagcaggtagatcaatctacaacagct tttatgtgtattgcaaaggcccctgtcaaagagtgcagccgggaaaactc agggtacagtgcagcacctgcaggcaggcaacgctcaccttgacccaggg tccatcttgctgggatgatgttttaattccaaaccggatgagtggtgaat gccaatccccacactgccctgggactagtgcagaatttttctttaaatgt ggagcacaccccacctctgacaaggaaacatcagtagctttgcacctgat cgcaacaaatagtcggaacatcacttgcattacgtgcacagacgtcagga gccccgtcctggttttccagtgcaactcccgccacgtgatttgcttagac tgtttccacttatactgtgtgacaagactcaatgatcggcagtttgttca

cgaccctcaacttggctactccctgccttgtgtggctggctgtcccaact
ccttgattaaagagctccatcacttcGCgattctgggagaagagcagtac
aaccggtaccagcagtatggtgcagaggagtgtgtcctgcagatgggggg
cgtgttatgcccccgccctggctgtggagcggggctgctgccggagcctg
accagaggaaagtcacctgcgaaggggcaatggcctgggctgtgggttt
gccttctgccgggaatgtaaagaagcgtaccatgaaggggagtgcagtgc
cgtatttgaagcctcaggaacaactactcaggcctacagagtcgatgaaa
gagccgccgagcaggctcgttgggaagcagcctccaaagaaaccatcaag
aaaaccaccaagccctgtccccgctgccatgtaccagtggaaaaaaatgg
aggctgcatgcacatgaagtgtccgcagccccagtgcaggctcgagtggt
gctggaactgtggctgcgagtggaaccgcgtctgcatgggggaccactgg
ttcgacgtgtaggcggccgc