Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin 80-465 [R305A]

Enzyme description:- Parkin 80-465 [R305A]

Clone number:- DU46036

Source:- BL21 recombinant

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

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

Expression level:- 1mg/L

Calculated molecular mass:-

Monoisotopic	42406Da
Average Mass	42433Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.92

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 80-465 [R305A]

Protein	Parkin 80-465 [R305A]
Synonyms	PARK2, PRKN2
Clone Number	DU46036
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**MNATGGDDPRNAAGGCEREPQSLTRVDLSSSVLPGDSVGLAVILHT DSRKDSPPAGSPAGRSIYNSFYVYCKGPCQRVQPGKLRVQCSTCRQATLT LTQGPSCWDDVLIPNRMSGECQSPHCPGTSAEFFFKCGAHPTSDKETSVA LHLIATNSRNITCITCTDVRSPVLVFQCNSRHVICLDCFHLYCVTRLNDR QFVHDPQLGYSLPCVAGCPNSLIKELHHFAILGEEQYNRYQQYGAEECVL QMGGVLCPRPGCGAGLLPEPDQRKVTCEGGNGLGCGFAFCRECKEAYHEG ECSAVFEASGTTTQAYRVDERAAEQARWEAASKETIKKTTKPCPRCHVPV EKNGGCMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWFDV**
Native sequence	in bold
Protease cleavage	SENP1 treatment after GG of SUMO1
Cloning sites	
DNA sequence of cassette	CCATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCA ACTGAGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAAACTCAAAGT CATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTGAAAATGACAACAC ATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTTCCAATG AACTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATAC TCCAAAAGAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGG AACAAACGGGGGGaatgaatgcaactggaggcgacgaccccagaaacgcg gcgggaggctgtgagcgggagccccagagcttgactcgggtggacctcag cagctcagtcctcccaggagactctgtggggctggctgtcattctgcaca ctgacagcaggaaggactcaccaccagctggaagtccagcaggtagatca atctacaacagcttttatgtgtattgcaaaggcccctgtcaaagagtgca gccgggaaaactcagggtacagtgcagcacctgcaggcaggcaacgctca ccttgacccagggtccatcttgctgggatgatgttttaattccaaaccgg atgagtggtgaatgccaatccccacactgccctgggactagtgcagaatt tttctttaaatgtggagcacaccccacctctgacaaggaaacatcagtag ctttgcacctgatcgcaacaaatagtcggaacatcacttgcattacgtgc acagacgtcaggagccccgtcctggttttccagtgcaactcccgccacgt gatttgcttagactgtttccacttatactgtgtgacaagactcaatgatc ggcagtttgttcacgaccctcaacttggctactccctgccttgtgtggct ggctgtcccaactccttgattaaagagctccatcacttcGCgattctggg agaagagcagtacaaccggtaccagcagtatggtgcagaggagtgtgtcc tgcagatgggggcgtgttatgcccccgccctggctgtggagcggggctg ctgccggagcctgaccagaggaaagtcacctgcgaaggggcaatggcct gggctgtgggtttgccttctgccgggaatgtaaagaagcgtaccatgaag

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