Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin K27N

Enzyme description:- Parkin 1-465 K27N

Clone number:- DU39837

Source:- BL21 recombinant

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

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

Expression level:- 0.4mg/L

Calculated molecular mass:-

Monoisotopic	51602 Da
Average Mass	51635 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 K27N

Protein	Parkin 1-465 K27N
Synonyms	PARK2, PRKN2
Clone Number	DU39837
Species	Human
Accession Number	Protein: O60260 Gene: NM_004562.2
Tags	N-terminal His_6-SUMO1-tag (cleaved)
Aminoacid sequence of the expressed protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLK KLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGG **MIVFVRFNSSHGFPVEVDSDTSIFQLNEVVAKRQGVPADQLRVIFAGKELRN** **DWTVQNCDLDQQSIVHIVQRPWRKGQEMNATGGDDPRNAAGGCEREPQSLTR** **VDLSSSVLPGDSVGLAVILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQR** **VQPGKLRVQCSTCRQATLTLTQGPSCWDDVLIPNRMSGECQSPHCPGTSAEF** **FFKCGAHPTSDKETPVALHLIATNSRNITCITCTDVRSPVLVFQCNSRHVIC** **LDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNSLIKELHHFRILGEEQY** **NRYQQYGAEECVLQMGGVLCPRPGCGAGLLPEPDQRKVTCEGGNGLGCGFAF** **CRECKEAYHEGECSAVFEASGTTTQAYRVDERAAEQARWEAASKETIKKTTK** **PCPRCHVPVEKNGGCMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWFDV**
Native sequence	in bold
Protease cleavage	SENP1 treatment after GG of SUMO1
Cloning sites	BamH1 / Not1 (destroyed)
DNA sequence of insert	atgatagtgtttgtcaggttcaactccagccatggtttcccagtggaggtcg attctgacaccagcatcttccagctcaaTgaggtggttgctaagcgacaggg ggttccggctgaccagttgcgtgtgattttcgcagggaaggagctgaggaat gactggactgtgcagaattgtgacctggatcagcagagcattgttcacattg tgcagagaccgtggagaaaaggtcaagaaatgaatgcaactggaggcgacga ccccagaaacgcggcgggaggctgtgagcgggagccccagagcttgactcgg gtggacctcagcagctcagtcctcccaggagactctgtggggctggctgtca ttctgcacactgacagcaggaaggactcaccaccagctggaagtccagcagg tagatcaatctacaacagcttttatgtgtattgcaaaggcccctgtcaaaga gtgcagccgggaaaactcagggtacagtgcagcacctgcaggcaggcaacgc tcaccttgacccagggtccatcttgctgggatgatgttttaattccaaaccg gatgagtggtgaatgccaatccccacactgccctgggactagtgcagaattt ttctttaaatgtggagcacaccccacctctgacaaggaaacaccagtagctt tgcacctgatcgcaacaaatagtcggaacatcacttgcattacgtgcacaga cgtcaggagccccgtcctggttttccagtgcaactcccgccacgtgatttgc ttagactgtttccacttatactgtgtgacaagactcaatgatcggcagtttg ttcacgaccctcaacttggctactccctgccttgtgtggctggctgtcccaa ctccttgattaaagagctccatcacttcaggattctgggagaagagcagtac aaccggtaccagcagtatggtgcagaggagtgtgtcctgcagatgggggcg tgttatgccccgccctggctgtggagcggggctgctgccggagcctgacca gaggaaagtcacctgcgaaggggcaatggcctgggctgtgggtttgccttc tgccgggaatgtaaagaagcgtaccatgaaggggagtgcagtgccgtatttg aagcctcaggaacaactactcaggcctacagagtcgatgaaagagccgccga

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