Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of Parkin C431S

Enzyme description:- Parkin 1-465 C431S

Clone number:- DU39796

Source:- BL21 recombinant

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

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

Expression level:- 0.5mg/L

Calculated molecular mass:-

Monoisotopic	51590 Da
Average Mass	51623 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 7.21

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 C431S

Protein	Parkin 1-465 C431S
Synonyms	PARK2, PRKN2
Clone Number	DU39796
Species	Human
Accession Number	Protein: O60260 Gene: NM_004562.2
Tags	N-terminal His-SUMO1-tag (cleaved)
Aminoacid sequence of the expressed protein	MGHHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLK KLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTGG **MIVFVRFNSSHGFPVEVDSDTSIFQLKEVVAKRQGVPADQLRVIFAGKELRN** **DWTVQNCDLDQQSIVHIVQRPWRKGQEMNATGGDDPRNAAGGCEREPQSLTR** **VDLSSSVLPGDSVGLAVILHTDSRKDSPPAGSPAGRSIYNSFYVYCKGPCQR** **VQPGKLRVQCSTCRQATLTLTQGPSCWDDVLIPNRMSGECQSPHCPGTSAEF** **FFKCGAHPTSDKETSVALHLIATNSRNITCITCTDVRSPVLVFQCNSRHVIC** **LDCFHLYCVTRLNDRQFVHDPQLGYSLPCVAGCPNSLIKELHHFRILGEEQY** **NRYQQYGAEECVLQMGGVLCPRPGCGAGLLPEPDQRKVTCEGGNGLGCGFAF** **CRECKEAYHEGECSAVFEASGTTTQAYRVDERAAEQARWEAASKETIKKTTK** **PCPRCHVPVEKNGGSMHMKCPQPQCRLEWCWNCGCEWNRVCMGDHWFDV**
Native sequence	in bold
Protease cleavage	SENP1 treatment after GG of SUMO1
Cloning sites	BamH1 (destroyed), Not1
DNA sequence of insert	ATGATAGTGTTTGTCAGGTTCAACTCCAGCCATGGTTTCCCAGTGGAGGTCG ATTCTGACACCAGCATCTTCCAGCTCAAGGAGGTGGTTGCTAAGCGACAGGG GGTTCCGGCTGACCAGTTGCGTGTGATTTTCGCAGGGAAGGAGCTGAGGAAT GACTGGACTGTGCAGAATTGTGACCTGGATCAGCAGAGCATTGTTCACATTG TGCAGAGACCGTGGAGAAAAGGTCAAGAAATGAATGCAACTGGAGGCGACGA CCCCAGAAACGCGGCGGGAGGCTGTGAGCGGGAGCCCCAGAGCTTGACTCGG GTGGACCTCAGCAGCTCAGTCCTCCCAGGAGACTCTGTGGGGCTGGCTGTCA TTCTGCACACTGACAGCAGGAAGGACTCACCACCAGCTGGAAGTCCAGCAGG TAGATCAATCTACAACAGCTTTTATGTGTATTGCAAAGGCCCCTGTCAAAGA GTGCAGCCGGGAAAACTCAGGGTACAGTGCAGCACCTGCAGGCAGGCAACGC TCACCTTGACCCAGGGTCCATCTTGCTGGGATGATGTTTTAATTCCAAACCG GATGAGTGGTGAATGCCAATCCCCACACTGCCCTGGGACTAGTGCAGAATTT TTCTTTAAATGTGGAGCACACCCCACCTCTGACAAGGAAACATCAGTAGCTT TGCACCTGATCGCAACAAATAGTCGGAACATCACTTGCATTACGTGCACAGA CGTCAGGAGCCCCGTCCTGGTTTTCCAGTGCAACTCCCGCCACGTGATTTGC TTAGACTGTTTCCACTTATACTGTGTGACAAGACTCAATGATCGGCAGTTTG TTCACGACCCTCAACTTGGCTACTCCCTGCCTTGTGTGGCTGGCTGTCCCAA CTCCTTGATTAAAGAGCTCCATCACTTCAGGATTCTGGGAGAAGAGCAGTAC AACCGGTACCAGCAGTATGGTGCAGAGGAGTGTGTCCTGCAGATGGGGGGCG TGTTATGCCCCCGCCCTGGCTGTGGAGCGGGGCTGCTGCCGGAGCCTGACCA GAGGAAAGTCACCTGCGAAGGGGGCAATGGCCTGGGCTGTGGGTTTGCCTTC TGCCGGGAATGTAAAGAAGCGTACCATGAAGGGGAGTGCAGTGCCGTATTTG AAGCCTCAGGAACAACTACTCAGGCCTACAGAGTCGATGAAAGAGCCGCCGA

GCAGGCTCGTTGGGAAGCAGCCTCCAAAGAAACCATCAAGAAAACCACCAAG
CCCTGTCCCCGCTGCCATGTACCAGTGGAAAAAAATGGAGGCTCCATGCACA
TGAAGTGTCCGCAGCCCCAGTGCAGGCTCGAGTGGTGCTGGAACTGTGGCTG
CGAGTGGAACCGCGTCTGCATGGGGGACCACTGGTTCGACGTGTAGGTCGAC
GGCGCGGCCGCG