

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of Parkin**

<b><u>Enzyme description:-</u></b>	Parkin 1-465 = full length
<b><u>Clone number:-</u></b>	DU40847
<b><u>Source:-</u></b>	BL21 recombinant
<b><u>Tag:-</u></b>	cleaved from N-terminal His-SUMO1 tag
<b><u>Purification method:-</u></b>	Ni <sup>++</sup> -Sepharose, SENP-treatment, Ni <sup>++</sup> -depletion, SEC
<b><u>Expression level:-</u></b>	0.2mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	51606 Da
Average Mass	51639 Da
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	7.21
<b><u>Purity:-</u></b>	90%
<b><u>Enzyme storage buffer:-</u></b>	
50mM HEPES pH 8.2, 150mM NaCl, 20% glycerol, 1mM TCEP	
<b><u>Storage temperature:-</u></b>	-80°C
<b><u>Assay:-</u></b>	

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### Clone Data Sheet

#### Parkin

<b><u>Protein</u></b>	Parkin 1-465
<b><u>Synonyms</u></b>	PARK2, PRKN2
<b><u>Clone Number</u></b>	DU40847
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Protein: O60260      Gene: NM_004562.2
<b><u>Tags</u></b>	N-terminal His <sub>6</sub> -SUMO1-tag (cleaved)
Aminoacid sequence of the expressed protein	MGHHHHHSDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFVKVMTTHLK KLKESYCYRQGVPMNSLRFLFEGORIAADNHTPKELGMEEEDVIEVYQEQTGG <b>MI VFVRFNSSHGFPVEVSDTSIFQLKEVVAKRQGV PADQLRVI FAGKELRN</b> <b>DWTVQNCDL DQOSIVHIVQRPWRKGOEMNATGGDDPRNAAGGCERE PQSLTR</b> <b>VDLSSSVLP GDSVGLAVILHTDSRKDSPAGSPAGRSIYNSFYVYCKGPCQR</b> <b>VQPGKLRVQCSTCRQATLTLTQGPSCWDDVLI PNRMSGECQSPHCPGTSAEF</b> <b>FFKCGAHPTSDKETSVALHLIATNSRNITCITCTDVRSPVLV FQCNSRHVIC</b> <b>LDCFHLYCVTRLNDRQFVHDPQLGYS LPCVAGCPNSLIKELHHFRILGEEQY</b> <b>NRYQQYGAEECVLQMGVLCPRPGC GAGLLPEPDQRKVTCEGGNGLGCGFAF</b> <b>CRECKEAYHEGEC SAVFEASGTTTQAYRVD ERAAEQARWEAASKETIKKTTK</b> <b>PCPRCHVPVEKNGGCMHMKCPQPQCRLEWCWNCGEWNRVCMGDHWF DV</b>
Native sequence	in bold
Protease cleavage	SEN1 treatment after GG of SUMO1
Cloning sites	
<b><u>DNA sequence of insert</u></b>	ATGGGTCATCATCACCATCACCATTCTGACCAGGAGGCAAAACCTTCAACTG AGGACTTGGGGGATAAGAAGGAAGGTGAATATATTAACCTCAAAGTCATTGG ACAGGATAGCAGTGAGATTCACCTTCAAAGTGAAAATGACAACACATCTCAAG AAACTCAAAGAATCATACTGTCAAAGACAGGGTGTCCAATGAACTCACTCA GGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATACTCCAAAAGAACT GGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGGAACAAACGGGGGGA atgatagtggttgcaggttcaactccagccatgggttcccagtgagggtcg attctgacaccagcatcttccagctcaaggaggtggttgctaagcgacaggg ggttccggctgaccagttgctgtgattttcgcaggaaggagctgaggaat gactggactgtgcagaattgtgacctggatcagcagagcattgttcacattg tgcagagaccgtggagaaaaggtaagaaatgaatgcaactggaggcgacga cccagaaaacgcggcgggaggctgtgagcgggagccccagagcttgactcgg gtggacctcagcagctcagtcctcccaggagactctgtgggctggctgtca ttctgcacactgacagcaggaaggactcaccaccagctggaagtccagcagg tagatcaatctacaacagcttttatgtgtattgcaaaggcccctgtcaaga gtgcagccgggaaaactcaggtacagtgacagcactgcaggcaggcaacgc tcaccttgaccagggtccatcttgcctgggatgatgttttaattccaaaccg gatgagtggtgaatgccaatccccacactgccctgggactagtgagaat ttctttaaatgtggagcacaccccacctctgacaaggaaacatcagtagctt tgcacctgatcgcaacaaatagtcggaacatcacttgacattacgtgcacaga cgtaggagccccgtcctggtttccagtgcaactcccgccacgtgatttgc ttagactgttccacttatactgtgtgacaagactcaatgatcggcagtttg ttcacgacctcaacttggctactccctgccttgtgtggctggctgtccaa

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gcaggctcgttgggaagcagcctccaaagaaaccatcaagaaaaccaccaag  
ccctgtccccgctgccatgtaccagtggaaaaaaatggaggctgcatgcaca  
tgaagtgtccgcagccccagtcaggctcgagtggctggaactgtggctg  
cgagtggaaccgcgtctgcatgggggaccactggttcgacgtgtagggggcc  
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