

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000] C727S

Enzyme description:- LRRK2 [1 – 1000] C727S

Clone number:- DU 27828

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 138, 930.83 daltons

Average Mass 139, 020.62 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.52

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

MRC PPU REAGENTS

Clone Data Sheet

LRRK2 [1 – 1000] C727S

Protein LRRK2 [1 – 1000] C727S

Clone number DU 27828

Species Human

Accession number NM_198578.3

Tags N-terminal GST

Bacterially
expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFOGPLGSMASGSCQGC
EDEETLKKLIVRLNNVQEGKQIETLVQILEDLLVFTYSEHASKLFOG
KNIHVPLLI VLDSYMRVASVQQVGWSSLCKLIEVCPGTMQSLMGPQDV
GNDWEVLGVHQLILKMLTVHNASVNL SVIGLKTLDLLLTSGKITLLIL
DEESDIFMLIFDAMHSFPANDEVQKLGCKALHVLFERVSEEQLTEFVE
NKDYMILLSALTNFKDEEEIVLHVLHCLHSLAIPCNNVEVLMSGNVRC
YNIVVEAMKAFPMSERIQEVSCLLHRLTLGNFFNILVLNEVHEFVVK
AVQQYPENAALQISALSCLALLTETIFLNQDLEEKNEQENDDEGEED
KLFWLEACYKALTWHRKNKHVQEAACWALNNLLMYQNSLHEKIGDEDG
HFPAHREVMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKGI
HLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLDIMAAVVPKILT
VMKRHETSLPVQLEALRAILHFIVPGMPEESREDTEFHKLNMVKKQC
FKNDIHKLVLAALNRFIGNPGIQKCGLKVISSIVHFDALEMLSLEGA
MDSVLHTLQMYPPDQEIQCGLGLSLIGYLITKKNVFIGTGHELLAKILVS
SLYRFKDVAEIQTKGFQTI LAILKLSASF SKLLVHHSFDLVI FHQMS
NIMEQKDQQFLNLCKCFAKVAMDDYLKNVMLERACDQNSIMV**ESLL**
LLGADANQAKEGSSLICQVCEKESPKLVELLLNSGSREQDVRKALTI
SIGKGDSQIISLLRLRALDVANNSICLGGFCIGKVEPSWLGPLFPDK
TSNLRKQTNIASTLARMVIRYQMKSAVEEGTASGSDGNFSEDVLSKFD
EWTFIPDSSMDSVFAQSDDL DSEGSEGSFLVKKKSNSISVGEFYRDAV
LQRCSPNLQRHSNSLGPFDHEDLLKRKRKILSSDDSLRSSKQLQSHMR
HSDSISSLASEREYITSLDLSANELRDIDAL

Native sequence Amino acids M1 – L1000 of human LRRK2 (end residue E2527). Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220. The enzyme has a C727S mutation. Residue C727 is equivalent to S958 of the fusion protein

MRC PPU REAGENTS

Protease cleavage PreScission (LEVLFQGP) residues 221 – 228

Cloning sites *Bam*H1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

ggatccATGGCTAGTGGCAGCTGTTCAGGGGTGCGAAGAGGACGAGGAAACTCTGAAGAAGTTGATAGTCAGGCTGAAC
AATGTCCAGGAAGGAAAACAGATAGAAAACGCTGGTCCAAATCCTGGAGGATCTGCTGGTGTTCACGTACTCCGAGCAC
GCCTCCAAGTTATTTCAAGGCCAAAAATATCCATGTGCCTCTGTTGATCGTCTTGGACTCCTATATGAGAGTCGCGAGT
GTGCAGCAGGTGGGTTGGTCACTTCTGTGCAAATTAATAGAACTGTGCCAGGTACAATGCAAAGCTTAATGGGACCC
CAGGATGTTGGAAATGATTGGGAAGTCTTGGTGTTCACCAATTGATTCTTAAAATGCTAACAGTTCATAATGCCAGT
GTAAACTTGTTCAGTGATTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAATAACACCTTGCTGATATTGGAT
GAAGAAAGTGATATTTTCATGTTAATTTTTGATGCCATGCACTCATTTCAGCCAATGATGAAGTCCAGAAACTTGGA
TGCAAAGCTTTACATGTGCTGTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATG
ATATTGTTAAGTGCCTTAACAAAATTTAAAGATGAAGAGGAAATTTGTGCTTCATGTGCTGCATTGTTTACATTCCCTA
GCGATTCCCTTGCAATAATGTGGAAGTCTCATGAGTGGCAATGTTCAGGTGTTATAATATTGTGGTGGAAAGCTATGAAA
GCATTCCCTATGAGTGAAGAATTCAGAAGTGTGCTGTTTGTCTCCATAGGCTTACATTAGGTAATTTTTTCAAT
ATCCTGGTATTAACGAAGTCCATGAGTTTGTGGTGAAGCTGTGCAGCAGTACCCAGAGAATGCAGCATTGCAGATC
TCAGCGCTCAGCTGTTTGGCCCTCCTCACTGAGACTATTTCTTAAATCAAGATTTAGAGGAAAAGAATGAGAATCAA
GAGAATGATGATGAGGGGAAGAAGATAAATTTGTTTGGCTGGAAGCCTGTTACAAAGCATTAACTGGCATAAGAAAG
AACAAGCACGTGCAGGAGGCCGCATGCTGGGCACATAAATAATCTCCTTATGTACCAAAAACAGTTTACATGAGAAGATT
GGAGATGAAGATGGCCATTTCCAGCTCATAGGGAAGTGTGCTCTCCATGTGATGCATTCTTTCATCAAAGGAAGTT
TTCCAGGCATCTGCGAATGCATTGTCAACTCTCTTAGAACAAAATGTTAATTTTCAGAAAAATACTGTTATCAAAGGA
ATACACCTGAATGTTTTGGAGTTAATGCAGAAGCATATACATTCTCCTGAAGTGGCTGAAAGTGGCTGTAAAATGCTA
AATCATCTTTTTGAAGGAAGCAACACTTCCCTGGATATAATGGCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAA
CGTCATGAGACATCATTACCAGTGCAGCTGGAGGCGCTTCGAGCTATTTTACATTTTATAGTGCCTGGCATGCCAGAA
GAATCCAGGGAGGATACAGAATTTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTCAGAATGATATTCACAAA
CTGGTCTTAGCAGCTTTGAACAGGTTTATTGGAAATCCTGGGATTACAGAAATGTGGATTAAAAGTAATTTCTTCTATT
GTACATTTTCTGATGCATTAGAGATGTTATCCCTGGAAGGTGCTATGGATTTCAGTGTTCACACACTGCAGATGTAT
CCAGATGACCAAGAAATTCAGTGTCTGGGTTTAAAGTCTTATAGGATACTTGATTACAAAAGAAGAATGTGTTTCATAGGA
ACTGGACATCTGCTGGCAAAAATCTGGTTCAGCTTATAACCGATTTAAGGATGTTGCTGAAATACAGACTAAAGGA
TTTCAGACAATCTTAGCAATCCTCAAATTTGTCAGCATCTTTTTCTAAGCTGCTGGTGCATCATTTCATTTGACTTAGTA
ATATTTCCATCAAATGTCTTCCAATATCATGGAACAAAAGGATCAACAGTTTCTAAACCTCTGTTGCAAGTGTGTTGCA
AAAGTAGCTATGGATGATTACTTAAAAAATGTGATGCTAGAGAGAGCGTGTGATCAGAATAACAGCATCATGGTTGAA
TCTTTGCTTCTATTGGGAGCAGATGCCAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTTCAGGTATGTGAGAAAGAG
AGCAGTCCCAAATTTGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGTCTTAAAGGAGGCTGGCCCTGGATGTGGCCAACAATAGCATTTCG
CTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCTTTTATTTCCAGATAAGACTTCTAATTTAAGG
AAACAAACAAATATAGCATCTACACTAGCAAGAATGGTGTATCAGATATCAGATGAAAAGTGTGTGGAAGAAGGAACA
GCCTCAGGCAGCGATGGAAATTTTTCTGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCCTGACTCTTCT
ATGGACAGTGTGTTTGTCTCAAAGTGTGACCTGGATAGTGAAGGAAGTGAAGGCTCATTTCCTGTGAAAAAGAAATCT
AATTCATTTAGTGTAGGAGAATTTTACCGAGATGCCGTATTACAGCGTTGCTCACCAAATTTGCAAAGACATTCCAAT
TCCTTGGGGCCCATTTTTGATCATGAAGATTTACTGAAGCGAAAAAGAAAAATACTATCTTCAGATGATTCACACTCAGG
TCATCAAACCTTCAATCCCATATGAGGCATTTCAGACAGCATTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCA
CTAGACCTTTCAGCAAATGAACTAAGAGATATTGATGCCCTATagcgccgc