MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000]

Enzyme description:- LRRK2 [1 – 1000]

Clone number:- DU 27159

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic	138, 946.80 daltons
Average Mass	139, 036.69 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]

Protein	LRRK2 [1 – 1000]
Clone number	DU 27159
Species	Human
Accession number	NM_198578.3
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MASGSCQGC** **EEDEETLKKLIVRLNNVQEGKQIETLVQILEDLLVFTYSEHASKLFQG** **KNIHVPLLIVLDSYMRVASVQQVGWSLLCKLIEVCPGTMQSLMGPQDV** **GNDWEVLGVHQLILKMLTVHNASVNLSVIGLKTLDLLLTSGKITLLIL** **DEESDIFMLIFDAMHSFPANDEVQKLGCKALHVLFERVSEEQLTEFVE** **NKDYMILLSALTNFKDEEEIVLHVLHCLHSLAIPCNNVEVLMSGNVRC** **YNIVVEAMKAFPMSERIQEVSCCLLHRLTLGNFFNILVLNEVHEFVVK** **AVQQYPENAALQISALSCLALLTETIFLNQDLEEKNENQENDDEGEED** **KLFWLEACYKALTWHRKNKHVQEAACWALNNLLMYQNSLHEKIGDEDG** **HFPAHREVMLSMLMHSSSKEVFQASANALSTLLEQNVNFRKILLSKGI** **HLNVLELMQKHIHSPEVAESGCKMLNHLFEGSNTSLDIMAAVVPKILT** **VMKRHETSLPVQLEALRAILHFIVPGMPEESREDTEFHHKLNMVKKQC** **FKNDIHKLVLAALNRFIGNPGIQKCGLKVISSIVHFPDALEMLSLEGA** **MDSVLHTLQMYPDDQEIQCLGLSLIGYLITKKNVFIGTGHLLAKILVS** **SLYRFKDVAEIQTKGFQTILAILKLSASFSKLLVHHSFDLVIFHQMSS** **NIMEQKDQQFLNLCCKCFAKVAMDDYLKNVMLERACDQNNSIMVECLL** **LLGADANQAKEGSSLICQVCEKESSPKLVELLLNSGSREQDVRKALTI** **SIGKGDSQIISLLLRRLALDVANNSICLGGFCIGKVEPSWLGPLFPDK** **TSNLRKQTNIASTLARMVIRYQMKSAVEEGTASGSDGNFSEDVLSKFD** **EWTFIPDSSMDSVFAQSDDLDSEGSEGSFLVKKKSNSISVGEFYRDAV** **LQRCSPNLQRHSNSLGPIFDHEDLLKRKRKILSSDDSLRSSKLQSHMR** **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.

Protease cleavage PreScission (LEVLFQGP) residues 221 – 228

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

Nucleotide Sequence Of Insert

```
ggatccATGGCTAGTGGCAGCTGTCAGGGGTGCGAAGAGGACGAGGAAACTCTGAAGAAGTTGAT
AGTCAGGCTGAACAATGTCCAGGAAGGAAAACAGATAGAAACGCTGGTCCAAATCCTGGAGGATC
TGCTGGTGTTCACGTACTCCGAGCACGCCTCCAAGTTATTTCAAGGCAAAAATATCCATGTGCCT
CTGTTGATCGTCTTGGACTCCTATATGAGAGTCGCGAGTGTGCAGCAGGTGGGTTGGTCACTTCT
GTGCAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAGCTTAATGGGACCCCAGGATGTTGGAA
ATGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAAAATGCTAACAGTTCATAATGCCAGT
GTAAACTTGTCAGTGATTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAAAATCACCTT
GCTGATATTGGATGAAGAAAGTGATATTTTCATGTTAATTTTTGATGCCATGCACTCATTTCCAG
CCAATGATGAAGTCCAGAAACTTGGATGCAAAGCTTTACATGTGCTGTTTGAGAGAGTCTCAGAG
GAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATGATATTGTTAAGTGCGTTAACAAATTT
TAAAGATGAAGAGGAAATTGTGCTTCATGTGCTGCATTGTTTACATTCCCTAGCGATTCCTTGCA
ATAATGTGGAAGTCCTCATGAGTGGCAATGTCAGGTGTTATAATATTGTGGTGGAAGCTATGAAA
GCATTCCCTATGAGTGAAAGAATTCAAGAAGTGAGTTGCTGTTTGCTCCATAGGCTTACATTAGG
TAATTTTTTCAATATCCTGGTATTAAACGAAGTCCATGAGTTTGTGGTGAAAGCTGTGCAGCAGT
ACCCAGAGAATGCAGCATTGCAGATCTCAGCGCTCAGCTGTTTGGCCCTCCTCACTGAGACTATT
TTCTTAAATCAAGATTTAGAGGAAAAGAATGAGAATCAAGAGAATGATGATGAGGGGGAAGAAGA
TAAATTGTTTTGGCTGGAAGCCTGTTACAAAGCATTAACGTGGCATAGAAAGAACAAGCACGTGC
AGGAGGCCGCATGCTGGGCACTAAATAATCTCCTTATGTACCAAAACAGTTTACATGAGAAGATT
GGAGATGAAGATGGCCATTTCCCAGCTCATAGGGAAGTGATGCTCTCCATGCTGATGCATTCTTC
ATCAAAGGAAGTTTTCCAGGCATCTGCGAATGCATTGTCAACTCTCTTAGAACAAAATGTTAATT
TCAGAAAAATACTGTTATCAAAAGGAATACACCTGAATGTTTTGGAGTTAATGCAGAAGCATATA
CATTCTCCTGAAGTGGCTGAAAGTGGCTGTAAAATGCTAAATCATCTTTTTGAAGGAAGCAACAC
TTCCCTGGATATAATGGCAGCAGTGGTCCCCAAAATACTAACAGTTATGAAACGTCATGAGACAT
CATTACCAGTGCAGCTGGAGGCGCTTCGAGCTATTTTACATTTTATAGTGCCTGGCATGCCAGAA
GAATCCAGGGAGGATACAGAATTTCATCATAAGCTAAATATGGTTAAAAAACAGTGTTTCAAGAA
TGATATTCACAAACTGGTCCTAGCAGCTTTGAACAGGTTCATTGGAAATCCTGGGATTCAGAAAT
GTGGATTAAAAGTAATTTCTTCTATTGTACATTTTCCTGATGCATTAGAGATGTTATCCCTGGAA
GGTGCTATGGATTCAGTGCTTCACACACTGCAGATGTATCCAGATGACCAAGAAATTCAGTGTCT
GGGTTTAAGTCTTATAGGATACTTGATTACAAAGAAGAATGTGTTCATAGGAACTGGACATCTGC
TGGCAAAAATTCTGGTTTCCAGCTTATACCGATTTAAGGATGTTGCTGAAATACAGACTAAAGGA
TTTCAGACAATCTTAGCAATCCTCAAATTGTCAGCATCTTTTTCTAAGCTGCTGGTGCATCATTC
ATTTGACTTAGTAATATTCCATCAAATGTCTTCCAATATCATGGAACAAAAGGATCAACAGTTTC
TAAACCTCTGTTGCAAGTGTTTTGCAAAAGTAGCTATGGATGATTACTTAAAAAATGTGATGCTA
GAGAGAGCGTGTGATCAGAATAACAGCATCATGGTTGAATGCTTGCTTCTATTGGGAGCAGATGC
CAATCAAGCAAAGGAGGGATCTTCTTTAATTTGTCAGGTATGTGAGAAAGAGAGCAGTCCCAAAT
TGGTGGAACTCTTACTGAATAGTGGATCTCGTGAACAAGATGTACGAAAAGCGTTGACGATAAGC
ATTGGGAAAGGTGACAGCCAGATCATCAGCTTGCTCTTAAGGAGGCTGGCCCTGGATGTGGCCAA
CAATAGCATTTGCCTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCCTTTAT
TTCCAGATAAGACTTCTAATTTAAGGAAACAAACAAATATAGCATCTACACTAGCAAGAATGGTG
ATCAGATATCAGATGAAAAGTGCTGTGGAAGAAGGAACAGCCTCAGGCAGCGATGGAAATTTTTC
TGAAGATGTGCTGTCTAAATTTGATGAATGGACCTTTATTCCTGACTCTTCTATGGACAGTGTGT
TTGCTCAAAGTGATGACCTGGATAGTGAAGGAAGTGAAGGCTCATTTCTTGTGAAAAAGAAATCT
AATTCAATTAGTGTAGGAGAATTTTACCGAGATGCCGTATTACAGCGTTGCTCACCAAATTTGCA
AAGACATTCCAATTCCTTGGGGCCCATTTTTGATCATGAAGATTTACTGAAGCGAAAAAGAAAAA
TACTATCTTCAGATGATTCACTCAGGTCATCAAAACTTCAATCCCATATGAGGCATTCAGACAGC
ATTTCTTCTCTGGCTTCTGAGAGAGAATATATTACATCACTAGACCTTTCAGCAAATGAACTAAG
AGATATTGATGCCCTAtagcggccgc
```