MRC PPU REAGENTS

Standard Operating Procedure

Preparation of LRRK2 [1 - 1000] L728V

Enzyme description:- LRRK2 [1 – 1000] L728V

Clone number:- DU 27830

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic	138, 932.79 daltons
Average Mass	139, 022.66 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

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Clone Data Sheet

LRRK2 [1 – 1000] L728V

Protein	LRRK2 [1 – 1000] L728V
Clone number	DU 27830
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
NIMEQKDQQFLNLCCKCFAKVAMDDYLKNVMLERACDQNNSIMVECVL
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. The enzyme has a L728V mutation. Residue L728 is equivalent to V959 of the fusion protein.

Protease cleavage PreScission (<u>LEVLFQGP</u>) residues 221 – 228

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

Nucleotide Sequence Of Insert

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ggatccATGGCTAGTGGCAGCTGTCAGGGGTGCGAAGAGGACGAGGAAACTCTGAAGAAGTTGATAGTCAGGCTGAAC
AATGTCCAGGAAGGAAAACAGATAGAAACGCTGGTCCAAATCCTGGAGGATCTGCTGGTGTTCACGTACTCCGAGCACG
CCTCCAAGTTATTTCAAGGCAAAAATATCCATGTGCCTCTGTTGATCGTCTTGGACTCCTATATGAGAGTCGCGAGTGTG
CAGCAGGTGGGTTGGTCACTTCTGTGCAAATTAATAGAAGTCTGTCCAGGTACAATGCAAAGCTTAATGGGACCCCAGG
ATGTTGGAAATGATTGGGAAGTCCTTGGTGTTCACCAATTGATTCTTAAAATGCTAACAGTTCATAATGCCAGTGTAAACT
TGTCAGTGATTGGACTGAAGACCTTAGATCTCCTCCTAACTTCAGGTAAAATCACCTTGCTGATATTGGATGAAGAAAGT
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CATGTGCTGTTTGAGAGAGTCTCAGAGGAGCAACTGACTGAATTTGTTGAGAACAAAGATTATATGATATTGTTAAGTGC
GTTAACAAATTTTAAAGATGAAGAGGAAATTGTGCTTCATGTGCTGCATTGTTTACATTCCCTAGCGATTCCTTGCAATAA
TGTGGAAGTCCTCATGAGTGGCAATGTCAGGTGTTATAATATTGTGGTGGAAGCTATGAAAGCATTCCCTATGAGTGAA
AGAATTCAAGAAGTGAGTTGCTGTTTGCTCCATAGGCTTACATTAGGTAATTTTTTCAATATCCTGGTATTAAACGAAGTC
CATGAGTTTGTGGTGAAAGCTGTGCAGCAGTACCCAGAGAATGCAGCATTGCAGATCTCAGCGCTCAGCTGTTTGGCCC
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TGCTGGGCACTAAATAATCTCCTTATGTACCAAAACAGTTTACATGAGAAGATTGGAGATGAAGATGGCCATTTCCCAGC
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CTCTCTTAGAACAAAATGTTAATTTCAGAAAAATACTGTTATCAAAAGGAATACACCTGAATGTTTTGGAGTTAATGCAGA
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TCAGAATAACAGCATCATGGTTGAATGCGTTCTTCTATTGGGAGCAGATGCCAATCAAGCAAAGGAGGGATCTTCTTTAA
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ACGAAAAGCGTTGACGATAAGCATTGGGAAAGGTGACAGCCAGATCATCAGCTTGCTCTTAAGGAGGCTGGCCCTGGA
TGTGGCCAACAATAGCATTTGCCTTGGAGGATTTTGTATAGGAAAAGTTGAACCTTCTTGGCTTGGTCCTTTATTTCCAG
ATAAGACTTCTAATTTAAGGAAACAAACAAATATAGCATCTACACTAGCAAGAATGGTGATCAGATATCAGATGAAAAGT
GCTGTGGAAGAAGGAACAGCCTCAGGCAGCGATGGAAATTTTTCTGAAGATGTGCTGTCTAAATTTGATGAATGGACCT
TTATTCCTGACTCTTCTATGGACAGTGTGTTTGCTCAAAGTGATGACCTGGATAGTGAAGGAAGTGAAGGCTCATTTCTT
GTGAAAAAGAAATCTAATTCAATTAGTGTAGGAGAATTTTACCGAGATGCCGTATTACAGCGTTGCTCACCAAATTTGCA
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ATTCACTCAGGTCATCAAAACTTCAATCCCATATGAGGCATTCAGACAGCATTTCTTCTCTGGCTTCTGAGAGAGAATATA
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