

Certificate of Analysis

LRRK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-919, 14-919-K, 14-919M

Parent Lot # D12KP012N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal FLAG-tagged, recombinant, human LRRK2 amino acids 970-end, expressed by baculovirus in Sf21 insect cells. Purified by immunoaffinity chromatography. Purity 73% by SDS-PAGE and Coomassie blue staining. MW = 180kDa.

Specific Activity (Parent lot# D12KP012N): 147U/mg, where one unit of LRRK2, active activity is defined as 1nmol phosphate incorporated into 250μM ERMtide (RLGRDKYKTLRQIRQ) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.166mg/ml of enzyme in 50mM Tris/HCl pH7.4, 300mM NaCl, 20mM β-glycerophosphate, 0.25mM sodium orthovanadate, 10mM NaF, 150μg/ml FLAG peptide, 20% v/v glycerol, 1mM benzamidine, 0.2mM PMSF, 5mM DTT. Frozen solution.

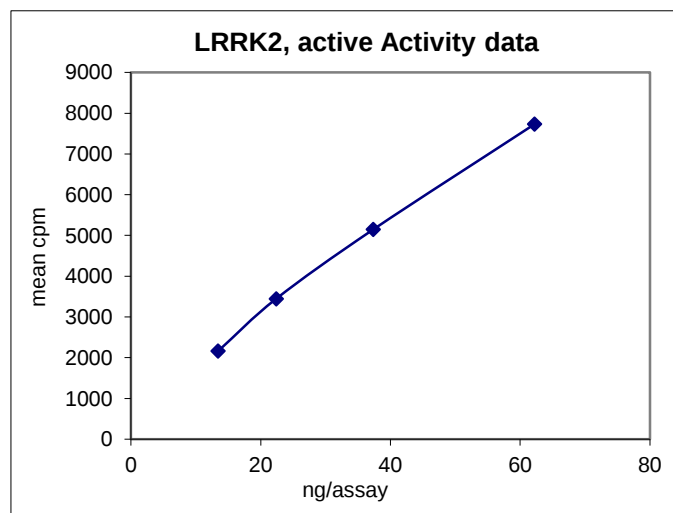
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

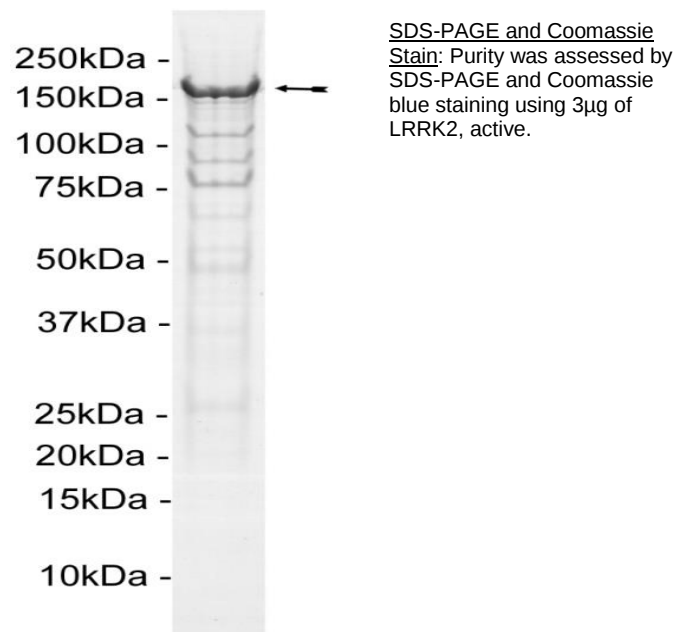
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 13–62ng of this lot of enzyme phosphorylated 250μM ERMtide (RLGRDKYKTLRQIRQ) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as LRRK2 with the translated sequence listed on page four.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 50mM HEPES pH8.0, 1mM EGTA, 0.01% Brij-35.
2. **BSA:** Use at a final assay concentration of 1%. Prepare a 10% stock and add 2.5µl of stock per assay point.
3. **DTT:** Use at a final assay concentration of 5mM. Prepare a 1M stock and add 0.125µl of stock per assay point.
4. **ERMtide (RLGRDKYKTLRQIRQ):** Use at a final assay concentration of 250µM. Prepare a 2.5mM stock and add 2.5µl of stock per assay point.
5. **LRRK2, active:** Dilute with 50mM HEPES pH8.0, 1mM EGTA, 0.01% Brij-35. Use 13–62ng per assay point.
6. **[γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 µl of 5 x reaction buffer per assay to wells.
2. Add 2.5 µl of ERMtide (RLGRDKYKTLRQIRQ)
3. Add **2.5 µl (13–62ng) LRRK2, active.**
4. Add 2.5 µl 10% BSA
5. Add 0.125 µl 1M DTT
6. Add 2.375 µl of dH₂O.
7. Add 10µl of diluted [γ -³³P]ATP mixture.
8. Incubate for 10 minutes at 30°C.
9. Stop the reaction by adding 5µl of 3% phosphoric acid.
10. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
11. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
12. Wash the filtermat once for 2 minutes with methanol.
13. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
14. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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LRRK2 Sequence Information

<u>Protein</u>	Human LRRK2
<u>Tags</u>	N-terminal FLAG
<u>Native sequence</u>	H27 of the recombinant protein is equivalent to H970 of human LRRK2
<u>Accession number</u>	GenBank BC117180

Recombinant LRRK2 amino acid sequence:

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1  MGGDYKDDDD KDRSHNQTSL YKKAGTHSDS ISSLASEREY ITSLDLSANE LRDIDALSQK
61  CCISVHLEHL EKLELHQNAL TSFPQQLCET LKSLTHLDLH SNKFTSFPSY LLKMSCIANL
121 DVSRNDIGPS VVLDPTVKCP TLKQFNLSYN QLSFVPENLT DVVEKLEQLI LEGNKISGIC
181 SPLRLKELKI LNLSKNHISS LSENFLEACP KVESFSARMN FLAAMPFLPP SMTILKLSQN
241 KFSCIPEAIL NPLHLRSLDM SSNDIQYLPG PAHWKSLNLR ELLFSHNQIS ILDLSEKAYL
301 WSRVEKLHLS HNKLKEIPPE IGCLENLTSL DVSYNLELRS FPNEMGKLSK IWDLPDELH
361 LNFDFKHIGC KAKDIIRFLQ QRLKKAVPYN RMKLMIVGNT GSGKTTLLQQ LMKTKKSDLG
421 MQSATVGIDV KDWPIQIRDK RKRDVLVNVW DFAGREEFYS THPHFMTQRA LYLAVIDLSK
481 GQAEVDAMKP WLFNIKARAS SSPVILVGTH LDVSDEKQRK ACMSKITKEL LNKRGFPAIR
541 DYHFVNATEE SDALAKLRKT IINESLNFKI RDQLVVGQLI PDCYVELEKI ILSERKNVPI
601 EFPVIDRKRL LQLVRENQLQ LDENELPHAV HFLNESGVLL HFQDPALQLS DLYFVEPKWL
661 CKIMAQILTV KVEGCPKHPK GIISRRDVEK FLSKKRKFPK NYMTQYFKLL EKFAQIALPIG
721 EEYLLVPSSL SDHRPVIELP HCENSEIIR LYEMPYFPMG FWSRLINRL EISPYMLSGR
781 ERALRPNRMY WRQGIYLNWS PEAYCLVGSE VLDNHPESFL KITVPSCRKG CILLGQVVDH
841 IDSLMEEWFP GLLIEDICGE GETLLKKWAL YSFNDGEEHQ KILLDDLMMK AEGDLLVNP
901 DQPRLTIPIS QIAPDLILAD LPRNIMLNND ELEFEQAPF LLGDGSFGSV YRAAYEGEEV
961 AVKIFNKHTS LRLLRQELVV LCHLHHPSLI SLLAAGIRPR MLVMELASKG SLDRLQLQDK
1021 ASLTRLQHR IALHVADGLR YLHSAMIIYR DLKPHNVLLF TLYPNAAIIA KIADYGIAQY
1081 CCRMGIKTSE GTPGFRAPEV ARGNVIYNQQ ADVYSFGLL YDILTGGRI VEGLKFPNEF
1141 DELEIQGKLP DPVKEYGCAP WPMVEKLIKQ CLKENPQERP TSAQVFDILN SAELVCLTRR
1201 ILLPKNVIVE CMVATHNSR NASIWLGCGR TDRGQLSFLD LNTEGYTSEE VADSRILCLA
1261 LVHLPVEKES WIVSGTQSGT LLVINTEDGK KRHTLEKMTD SVTCLYCNSF SKQSKQKNFL
1321 LVGTADGKLA IFEDKTVKLK GAAPLKILNI GNVSTPLMCL SESTNSTERN VMWGGCGTKI
1381 FSFSNDFTIQ KLIETRTSQL FSYAAFSDSN IITVVVDTAL YIAKQNSPVV EVWDKKTEKL
1441 CGLIDCVHFL REVTVKENKE SKHKMSYSGR VKTLCLQKNT ALWIGTGGGH ILLDLSTRR
1501 LIRVIYNFCN SVRVMMAQL GSLKNVMLVL GYNRKNTGEGT QKQKEIQSCL TVWDINLPHE
1561 VQNLEKHIEV RKELAEKMRR TSVE

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Recombinant LRRK2 nucleotide sequence:

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61  tacaaaaaag ccggcacgca ttcagacagc atttcttctc tggcttctga gagagaatat
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Reviewed and approved by site quality representative.

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