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Catalog #	Aliquot Size
L10-12GG-05	5 µg
L10-12GG-10	10 µg

LRRK2 (G2019S), Active

Recombinant human protein expressed in Sf9 cells

Catalog # L10-12GG

Lot # B1961-5

Product Description

Recombinant human LRRK2 (G2019S) (968-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The LRRK2 gene accession number is NM_198578.

Gene Aliases

PARK8; RIPK7; ROCO2; AURA17; DARDARIN

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, and 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

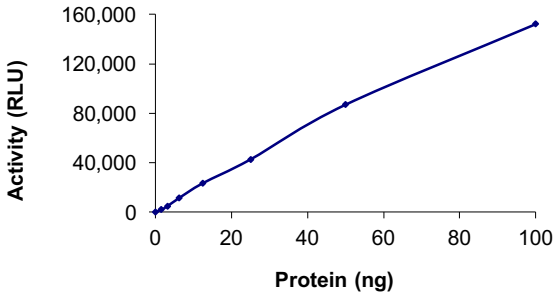
Scientific Background

LRRK2 or leucine-rich repeat kinase is a protein with an ankryin repeat region, a leucine-rich repeat (LRR) domain, a kinase domain, a DFG-like motif, a RAS domain, a GTPase domain, a MLK-like domain, and a WD40 domain. Mutations in LRRK2 are the most frequent known cause of autosomal dominant and idiopathic Parkinson's disease with prevalent mutations being found within the GTPase and kinase domains (1). LRRK2 cooperates with MET to promote efficient tumor cell growth and survival in various cancers. Down-regulation of LRRK2 in cultured tumor cells compromises MET activation and selectively reduces downstream MET signaling to mTOR and STAT3 (2).

References

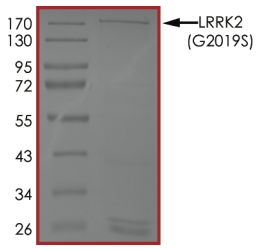
1. Yao C, LRRK2-mediated neurodegeneration and dysfunction of dopaminergic neurons in a Caenorhabditis elegans model of Parkinson's disease. Neurobiol Dis. 2010 Oct;40(1):73-81.
2. Looyenga BD, Chromosomal amplification of leucine-rich repeat kinase-2 (LRRK2) is required for oncogenic MET signaling in papillary renal and thyroid carcinomas. Proc Natl Acad Sci U S A. 2011 Jan 25;108(4):1439-44.

Specific Activity



The specific activity of LRRK2 (G2019S) was determined to be **5.4 nmol/min/mg** as per activity assay protocol, and was equivalent to **18 nmol/min/mg** as per radiometric assay.

Purity



The purity of LRRK2 (G2019S) was determined to be **>70%** by densitometry, approx. MW **210 kDa**.

LRRK2 (G2019S), Active

Recombinant human protein expressed in Sf9 cells

Catalog #	L10-12GG
Specific Activity	5.4 nmol/min/mg
Lot #	B1961-5
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: L10-12GG)

Active LRRK2 (G2019S) (0.1μg/μl) diluted with Kinase Dilution Buffer X (1x) (Catalog #: K20-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of active LRRK2 (G2019S) for optimal results).

Kinase Assay Buffer III (5x) (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH 7.4, 100mM $MgCl_2$ and 0.5mg/ml BSA. Add fresh DTT prior to use to a final concentration of 250μM.

Kinase Dilution Buffer IX (1x) (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with cold water. Add fresh DTT to the aliquot prior to use to a final concentration of 50μM.

ADP-GloTM Kinase Assay Kit (Promega, Cat # V9101)

ATP solution, 10 mM
ADP solution, 10 mM
ADP-Glo™ Reagent
Kinase Detection Reagent

Substrate (Catalog #: L10-58)

LRRKtide (RLGRDKYKTLRQIRQ) diluted in distilled H_2O to a final concentration of 0.2mg/ml.

Assay Protocol

The LRRK2 (G2019S) assay is performed using the ADP-Glo™ Kinase Assay kit (Promega; Cat# V9101) which quantifies the amount of ADP produced by the LRRK2 (G2019S) reaction. The ADP- Glo™ Reagent is added to terminate the kinase reaction and to deplete the remaining ATP, and then the Kinase Detection Reagent is added to convert ADP to ATP and to measure the newly synthesized ATP using luciferase/luciferin reaction.

Step 1. Thaw the Active LRRK2 (G2019S), Kinase Assay Buffer III (5x), and Substrate on ice. Prepare a 15 μL enzyme dilution at the desired concentration, with Kinase Dilution Buffer IX (1x), in a pre-chilled 96-well plate.

Step 2. Prepare a substrate/ATP mixture as follows (25 μM example):

Component	Amount (μL)	Component	Amount (μL)
10μM ATP Solution	1	Substrate at 1mg/mL	80
Kinase Assay Buffer III (5x)	79		

Step 3. Transfer the following reaction components prepared in Step 2 to a 384-well opaque plate bringing the reaction volume up to 5μL:

Component 1. 3μl of diluted Active LRRK2 (G2019S) (Catalog # L10-12GG).
Component 2. 2μl of Substrate/ATP mix as prepared in the table above. This initiates the reaction.

Step 4. Set up the blank control as outlined in step 2, excluding the addition of the kinase. Replace the kinase with an equal volume of Kinase Dilution Buffer IX (1x).

Step 5. Incubate at ambient temperature for 40 minutes.

Step 6. After the 40-minute incubation period, terminate the reaction and deplete the remaining ATP by adding 5μl of ADP-Glo™ Reagent. Spin down and shake the 384-well plate. Then incubate the reaction mixture for another 40 minutes at ambient temperature.

Step 7. Then add 10μl of the Kinase Detection Reagent to the 384-well plate and incubate the reaction mixture for another 30 minutes at ambient temperature.

Step 8. Read the 384-well reaction plate using the Luminescence Module Protocol on a GloMax®-Multi Microplate Multimode Reader (Promega; Cat# E7061).

Step 9. Determine the corrected activity (RLU) by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of Specific Activity of ADP (RLU/pmol)

From ADP standard curve, determine RLU/pmol of ADP

Kinase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected RLU from reaction / [(SA of ADP in RLU/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)

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