



AUG 25, 2023

In vitro LRRK2 autophosphorylation

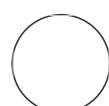
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ABSTRACT

This protocol details methods for the *in vitro* LRRK2 autophosphorylation assay.

ATTACHMENTS

[iuubbv9p.docx](#)

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Protocol status: Working
We use this protocol and it's working

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Keywords: LRRK2, Autophosphorylation, In vitro


MATERIALS

 Prescission Protease Genscript Catalog #Z02799

Glutathione beads (GE Healthcare, [17075601](#)),

 Amicon Ultra-15 Centrifugal Filter Unit Millipore
Sigma Catalog #UFC901024 & UFC903024

 Slide-A-Lyzer™ MINI Dialysis Device, 10K MWCO, 0.1 mL Thermo
Fisher Catalog #69572

 Anti-LRRK2 (phospho T1357)
antibody Abcam Catalog #ab270606

Solutions to prepare:




10x Kinase buffer:

A	B
Tris-HCl (pH7.5)	200 mM
MgCl ₂	75 mM
EGTA	1 mM

Dialysis buffer

A	B
HEPES (7.4)	20 mM
NaCl	150 mM
MgCl ₂	2.5 mM
Glycerol	5%
DTT	2 mM
GDP	20 μM

In vitro LRRK2 autophosphorylation

- 1 Set up the reaction mixture in a 1.7 mL Eppendorf tube with  1.4 mL purified LRRK2 protein, 1x kinase buffer with  1 millimolar (mM) ATP and  0.01 U/μL GST-Prescission Protease (to remove the Flag tag).

Note



Note: the LRRK2 protein used in this experiment was obtained by elution from the anti-FLAG M2 resin as described in the LRRK2 purification protocol.

- 2 Incubate samples  Overnight at  4 °C .



- 3 Add Glutathione beads to remove GST-Prescission Protease.



- 4 Concentrate samples by centrifugal filters and dialyze  Overnight at  4 °C against dialysis buffer.



- 5 Check autophosphorylation by Western blotting using a LRRK2 phospho-specific (pT1357) antibody.

- 6 Determine protein concentration by SDS-PAGE using Bovine Serum Albumin (BSA) as standard and used without freezing in the liposome tubulation experiments.