AUG 25, 2023

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# In vitro LRRK2 autophosphorylation

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ABSTRACT

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

ATTACHMENTS

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**DOI:** dx.doi.org/10.17504/protocols.io.81wgb6m91lpk/v1

**Protocol Citation:** Xinbo Wang, Pietro De Camilli 2023. In vitro LRRK2 autophosphorylation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgb6m91lpk/v1



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

**Created:** Aug 19, 2022

**Last Modified:** Aug 25, 2023

**PROTOCOL integer ID:** 68881

**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 |
| | MgCl2 | 75 mM |
| | EGTA | 1 mM |

**Dialysis buffer**

| | A | B |
|---|---|---|
| | HEPES (7.4) | 20 mM |
| | NaCl | 150 mM |
| | MgCl2 | 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 [M] 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.