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In vitro LRRK2 autophosphorylation

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

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Abstract

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

Attachments

DOCX

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18KB

Materials

Prescission Protease **Genscript Catalog #**Z02799

Glutathione beads (GE Healthcare, 17075601),

Amicon Ultra-15 Centrifugal Filter Unit **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**UFC901024 & UFC903024

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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.