***In vitro*** **LRRK2 autophosphorylation**

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

Materials: GST-Prescission Protease (GenScript, Z02799); Glutathione beads (GE Healthcare, 17075601); Centrifugal filters (Sigma, UFC901024); Mini dialysis units (Thermo Scientific, 69572); Anti-LRRK2 (phospho T1357) antibody (Abcam, ab270606).

**Solutions to prepare:**

10x kinase buffer: 200 mM Tris–HCl (pH7.5), 75 mM MgCl2 and 1 mM EGTA.

Dialysis buffer: 20mM HEPES 7.4, 150 mM NaCl, 2.5 mM MgCl2, 5% Glycerol, 2mMDTT, 20μM GDP

**Protocol:**

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 mM ATP and 0.01U/ul GST-Prescission Protease (to remove the Flag tag).

**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.