**LRRK2 expression and purification**

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**Abstract:** This protocol details methods for the expression of human LRRK2 in Expi293F cells and its *in vitro* purification.

Materials: ExpiFectamine293 Transfection Kit (Thermo Scientific, A14525); Prescission Protease (GenScript, Z02799); 3xFLAG Peptide (Sigma, F4799); Protease inhibitor cocktail (Roche, 05056489001); Glutathione Sepharose (GE Healthcare, 17075601); Mini dialysis units (Thermo Scientific, 69572); Centrifugal filters units (Sigma, UFC901024); Monoclonal ANTI-FLAG M2 resin (Sigma, F3165); EDTA-free protease inhibitor cocktail (Roche).

Solutions to prepare:

Lysis buffer: 20mM HEPES 7.4, 500mM NaCl, 10% Glycerol, 2mM DTT and 1xcomplete EDTA-free protease inhibitor.

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

**Protocol:**

1, Constructs encoding 3xFlag-LRRK2, 3xFlag-LRRK2(I2020T), 3xFlag-RCKW or 3xFlag-GFP-LRRK2 were transfected into Expi293F cells according to manufacturer instructions.

2, Proteins were expressed for three days following induction according to manufacturer instructions.

3, Cells were harvested by centrifugation (400xg, 4min) and lysed by 3 freeze-thaw cycles in lysis buffer.

**Note:** For 60ml of cell suspension, we used 15ml lysis buffer.

4, Cellular debris were removed by centrifugation at 15000xg for 1 hour at 4°C.

5, Clarified lysate was mixed with anti-FLAG M2 resin for 2 h while rotating at 4°C.

**Note:** For 60ml of cell suspension, we used 180ul of Anti-FLAG resin.

6, The resin was then washed with 3x10 bed volumes of lysis buffer.

7, Proteins were eluted with lysis buffer supplemented with 0.2 mg/mL 3xFlag peptides.

**Note:** For 60 ml of cell suspension, we used 800 µL elution buffer (without protease inhibitor)

8, The N-terminal 3xFlag tag was removed by incubation with the GST tagged Prescission Protease (0.01U/µl) overnight while rotating at 4°C.

9, The GST tagged Prescission Protease was subsequently removed by Glutathione Sepharose.

10, The purity of the proteins was assessed by SDS-PAGE and Western blotting.

11, Purified proteins were dialyzed overnight at 4°C against the dialysis buffer.

12, After dialysis, proteins were further clarified by centrifugation at 17000xg for 10 min at 4°C

13, Protein concentration was determined by SDS-PAGE using Bovine Serum Albumin (BSA) as standard and used without freezing in liposome binding and tubulation experiments.