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# LRRK2 expression and purification

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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 expression of human LRRK2 in Expi293F cells and its *in vitro* purification.

## Attachments

DOCX

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20KB

## Materials

ExpiFectamine™ 293 Transfection Kit **Thermo Fisher Catalog #**A14525

Prescission Protease **Genscript Catalog #**Z02799

3xFLAG Peptide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F4799

cOmplete™, EDTA-free Protease Inhibitor Cocktail **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**05056489001

Glutathione Sepharose (GE Healthcare, 17075601)

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

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

Monoclonal ANTI-FLAG M2 resin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F3165

EDTA-free protease inhibitor cocktail (Roche).

**Solutions to prepare:**

**Lysis salt buffer:**

| | A | B |
|---|---|---|
| | HEPES (7.4) | 20 mM |
| | NaCl | 500 mM |
| | Glycerol | 10 % |
| | DTT | 2 mM |
| | 1xcomplete EDTA-free protease inhibitor | |

**Dialysis buffer:**

| | A | B |
|---|---|---|
| | HEPES (7.4) | 20 mM |
| | NaCl | 150 mM |
| | Glycerol | 5 % |
| | MgCl2 | 2.5 mM |
| | DTT | 2 mM |
| | GDP | 20 µM |

## LRRK2 expression and purification

3h 14m

1 Transfect the constructs encoding 3xFlag-LRRK2, 3xFlag-LRRK2(I2020T), 3xFlag-RCKW or 3xFlag-GFP-LRRK2 into Expi293F cells according to manufacturer instructions.

2 Express the proteins for 72:00:00 following induction according to manufacturer instructions. 3d

3 Harvest the cells by centrifugation ( 400 x g , 00:04:00 ) and lyse by 3 freeze-thaw cycles in lysis buffer. 4m

Note

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

4 Remove the cellular debris by centrifugation at 15000 x g for 01:00:00 at 4 °C . 1h

5 Mix the clarified lysate with anti-FLAG M2 resin for 02:00:00 while rotating at 4 °C . 2h

Note

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

6 Wash the resin with 3×10 bed volumes of lysis buffer.

7 Elute the proteins with lysis buffer supplemented with [M] 0.2 mg/mL 3xFlag peptides.

Note

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

8 Remove the N-terminal 3xFlag tag by incubation with the GST tagged Prescission Protease ( 0.01 U/µL ) Overnight while rotating at 4 °C .

9 Remove the GST tagged Prescission Protease subsequently by Glutathione Sepharose.

10 Assess the purity of the proteins by SDS-PAGE and Western blotting.

11 Dialyze the purified proteins Overnight at 4 °C against the dialysis buffer.

12 After dialysis, clarify the proteins by centrifugation at 17000 x g for 00:10:00 at 4 °C . 10m

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