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

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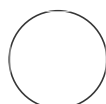
## LRRK2 expression and purification

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### ABSTRACT

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

### ATTACHMENTS

[iut8bvk9p.docx](#)

### MATERIALS

ExpiFectamine™ 293 Transfection Kit Thermo  
 Fisher Catalog #A14525

Prescission Protease Genscript Catalog #Z02799

3xFLAG Peptide Sigma –  
 Aldrich Catalog #F4799

cOmplete™, EDTA-free Protease Inhibitor Cocktail 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 Millipore  
 Sigma Catalog #UFC901024 & UFC903024

Monoclonal ANTI-FLAG M2 resin Millipore  
 Sigma Catalog #F3165

EDTA-free protease inhibitor cocktail (Roche).

### Solutions to prepare:

**PROTOCOL integer ID:**  
68877

**Keywords:** LRRK2, Expi293F cells, In vitro purification

**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 %
MgCl <sub>2</sub>	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 3x10 bed volumes of lysis buffer.






7

Elute the proteins with lysis buffer supplemented with  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.