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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

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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:

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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 %
	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 3x10 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.