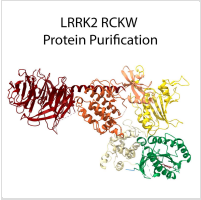

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LRRK2 RCKW Protein Purification

David Snead[1,2], Yu Xuan Lin[1], Mariusz Matyszewski[1]

[1]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093;

[2]Department of Biochemistry and Molecular Biology, Johns Hopkins University Bloomberg School of Public Heath, Baltimore, MD, 21205

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Mariusz Matyszewski

Protein purification protocol for tag-less LRRK2^{RCKW} as done by Leschziner and Reck-Peterson Labs. Same protocol can be used to purify LRRK1^{RCKW} as well.

Original Protocol by David Snead. Modified by Yu Xuan Lin and Mariusz Matyszewski for publication.

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Equipment:
Ultracentrifuge, we used one with a Ti70 rotor
Dounce homogenizer
FPLC with SP, His, and S200I columns.
Also make sure Ni-NTA beads and TEV protease are available.

Buffers (suggested volumes listed):
Day 1:

300 mL Lysis Buffer :

[M]50 millimolar (mM) HEPES **pH7.4**

[M]0.5 Molarity (M) NaCl

[M]20 millimolar (mM) Imidazole **pH8.0**

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

Plus protease inhibitors (Protein inhibitor tablets and **[M]0.5 millimolar (mM) Pefabloc**)

300 mL Wash Buffer :

[M]50 millimolar (mM) HEPES **pH7.4**

[M]0.5 Molarity (M) NaCl

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

100 mL Elution Buffer :

[M]50 millimolar (mM) HEPES **pH7.4**

[M]0.5 Molarity (M) NaCl

[M]300 millimolar (mM) Imidazole **pH8.0**

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

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300 mL Dilution Buffer :

[M]50 millimolar (mM) HEPES pH7.4

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

500 mL Buffer A (250 mM NaCl) :

[M]50 millimolar (mM) HEPES pH7.4

[M]250 millimolar (mM) NaCl

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

300 mL Buffer B (2.5 M NaCl) :

[M]50 millimolar (mM) HEPES pH7.4

[M]2.5 Molarity (M) NaCl

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

Day 2:

500 mL Buffer 0 :

[M]50 millimolar (mM) HEPES pH7.4

[M]0.5 Molarity (M) NaCl

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

300 mL Buffer 300 :

[M]50 millimolar (mM) HEPES pH7.4

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[M]0.5 Molarity (M) NaCl

[M]300 millimolar (mM) Imidazole pH8.0

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

300 mL Storage Buffer :

[M]20 millimolar (mM) HEPES pH7.4

[M]0.7 Molarity (M) NaCl

[M]0.5 millimolar (mM) TCEP

[M]5 % volume Glycerol

[M]2.5 millimolar (mM) MgCl2

[M]20 micromolar (µM) GDP

Day 1: Protein Pellet Lysis 1h 28m

1 Ready the Ultracentrifuge and cool it down to 4 °C

2 Make **Lysis Buffer** (check Materials section)

3 Thaw protein pellets On ice

4 Resuspend each pellet in 40 mL **Lysis Buffer**

> Might need to use a spatula and a pipette to resuspend fully.

5 Homogenize each pellet with Dounce homogenizer On ice

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5.1 15 plunges loose, followed by 17 plunges tight.

Gentle when homogenizing, don't completely pull douncer out of liquid, avoid bubbles

6 Pour lysate into rotor tubes (pre-chilled, clean, check for cracks; we used Ti70 tubes)

6.1 Make sure they are balanced. Use Lysis buffer to balance if needed.

7 Spin lysate in ultracentrifuge **50000 rpm, 4°C, 01:28:00 , Ti70 rotor** 1h 28m

8 Prep the rest of Day 1 buffers while waiting

Day 1: Ni-NTA gravity column 2m

9 Start equilibrating **12 mL** of Ni-NTA beads with **Lysis buffer** when there is about 30 mins left on the centrifuge

9.1 We equilibrated by resuspending **6 mL** in a 15 mL falcon tube, twice. 2m
They were spun down at **1000 rpm, 4°C, 00:02:00 , 3 times** , each time getting rid of liquid and doing a 50:50 resuspension.

10 After ultracentrifuge is done, pour the supernate into 3x 50 mL falcon tubes.

11 Add **4 mL equilibrated Ni-NTA beads** to 40 mL of supernate. Bring up to **50 mL with Lysis buffer**

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12 Incubate in cold room, while rotating "hot-dog style".
0 rpm, 4°C, 01:00:00 medium-slow rotation

13 After incubation, add onto a gravity column. Wash with remaining **Lysis Buffer**, followed by **200 mL Wash Buffer**

14 Elute with **50 mL Elution Buffer**
Make sure to resuspend the beads with stopper closed on the column. Might need multiple repeats of resuspension for best results. Leave column dry afterwards.

15 Dilute the solution to **250 millimolar (mM) NaCl** by adding **50 mL Dilution Buffer**

16 Syringe filter the solution.

Day 1: FPLC Cation Exchange and Overnight TEV cleavage

17 Use a 5 mL HiTrap SP FF column. Pump wash FPLC with **Buffer A** and **Buffer B**. Equilibrate the column with **Buffer A**.

18 Apply sample onto the column with a sample pump

19 Run a gradient program, **250 millimolar (mM) NaCl** to **2.5 Molarity (M) NaCl** , 0% B to 100% B.

20 Combine protein fractions into a 50 mL Falcon Tube. Check protein concentration; should be around **1.1 micromolar (µM)**

20.1 Afterwards dilute to **600 millimolar (mM) NaCl**

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20.2 Bring up to **50 mL with Wash Buffer**

21 Add TEV, and incubate overnight at **4 °C** while rotating "hot-dog style".

Our final TEV concentration was around **0.2 micromolar (µM)**

Day 2: FPLC HisTrap Column

22 Make Day 2 buffers. Make sure to degas the **Storage Buffer**.

23 Put the 5 mL HisTrap column on the FPLC. Pump wash the FPLC with **Buffer 0** and **Buffer 300**. Equilibrate the column with **Buffer 0**.

24 Run a Step Gradient Program

0% for 4 CV
6.7% for 5 CV
13.3% for 5 CV
26.7% for 5 CV
50% for 4 CV
100% for 6 CV (Column Wash)
0% for 5 CV (Column Re-equilibration)

25 Combine protein elution into a 15 mL tube before concentrating for Size Exclusion Column

Day 2: SEC Column 2m

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26 Equilibrate a S200I 10/300 column with the **Storage Buffer**.

27 Concentrate the protein to **1 mL** using 100 kD cutoff concentrators. Should be done nearing the end of the column equilibration.

We concentrated using 2 concentrators to save time.

27.1 After concentrating, filter the protein using a 0.1 uM spin filter. Spin at max[2m] speed for **00:02:00** at **4 °C**

28 Inject filtered sample and run elution program.

29 Collect and concentrate the protein to about **500 µL** . Concentration should be around **25-30 micromolar (µM)** after concentrating.

About 3-6 mL of protein initially.

30 Aliquot and freeze protein. Make sure to check pureness of protein as well.

2, 5, and/or 10 uL aliquots recommended.

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