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LRRK1 expression and purification

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We use this protocol and it's working!

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Abstract

Protein purification protocol for full-length LRRK1 as done by Leschziner and Reck-Peterson Labs.

Original protocol by Janice M. Reimer and Yu Xuan Lin.

Materials

Equipment:
Ultracentrifuge, we used one with a Ti70 rotor
Dounce homogenizer
FPLC with SP, His, and S200l columns.
Also make sure Ni-NTA beads and TEV protease are available.

Buffers (suggested volumes listed):
Day 1:

300 mL Lysis Buffer :
50 millimolar (mM) HEPES 7.4
0.5 Molarity (M) NaCl
20 millimolar (mM) Imidazole 8.0
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP
Plus protease inhibitors (Protein inhibitor tablets and 0.5 millimolar (mM) Pefabloc)

300 mL Wash Buffer :
50 millimolar (mM) HEPES 7.4
0.5 Molarity (M) NaCl
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

100 mL Elution Buffer :
50 millimolar (mM) HEPES 7.4
0.5 Molarity (M) NaCl
300 millimolar (mM) Imidazole 8.0
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

300 mL Dilution Buffer :

50 millimolar (mM) HEPES 7.4
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

500 mL Buffer A (250 mM NaCl) :
50 millimolar (mM) HEPES 7.4
250 millimolar (mM) NaCl
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

300 mL Buffer B (2.5 M NaCl) :
50 millimolar (mM) HEPES 7.4
2.5 Molarity (M) NaCl
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

Day 2:

500 mL Buffer 0 :
50 millimolar (mM) HEPES 7.4
0.5 Molarity (M) NaCl
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

300 mL Buffer 300 :
50 millimolar (mM) HEPES 7.4
0.5 Molarity (M) NaCl
300 millimolar (mM) Imidazole 8.0
0.5 millimolar (mM) TCEP
5 % volume Glycerol
5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

300 mL Storage Buffer ：
20 millimolar (mM) HEPES 7.4
0.7 Molarity (M) NaCl
0.5 millimolar (mM) TCEP
5 % volume Glycerol
2.5 millimolar (mM) MgCl2
20 micromolar (µM) GDP

Troubleshooting

His6-Z-TEV-LRRK1 Purification

4d 6h

1	N-terminally tagged His6-Z-TEV-LRRK1(FL) was expressed in Sf9 insect cells. Insect cells were infected with baculovirus and grown at 27°C for 3 days.	3d
2	Cells were harvested and then resuspended in lysis buffer (50mM HEPES pH 7.4, 500 mM NaCl, 20 mM imidazole, 0.5 mM TCEP, 5% glycerol, 5 mM MgCl2, 20 µM GDP, 0.5 mM Pefabloc and cOmplete EDTA-free protease inhibitor cocktail (Roche).	1h
3	Cells were lysed using a Dounce homogenizer and clarified by centrifugation at 50000 rpm, 4°C, 01:28:00, and Ti70 rotor.	2h
4	The supernatant was incubated for 1 hr with Ni-NTA agarose beads (Qiagen) equilibrated in lysis buffer. Beads were applied to a gravity column where they were extensively washed with lysis buffer, wash buffer (50mM HEPES pH 7.4, 500mM NaCl, 0.5mM TCEP, 5% glycerol, 5mM MgCL2, 20uM GDP), followed by elution in lysis buffer containing 300 mM imidazole (50mM HEPES pH 7.4, 500mM NaCl, 0.5mM TCEP, 5% glycerol, 5mM MgCL2, 20uM GDP, 300mM imidazole)	2h
5	The eluted protein was diluted to 250 mM NaCl using dilution buffer (50mM HEPES pH 7.4, 0.5mM TCEP, 5% glycerol, 5mM MgCL2, 20uM GDP) and loaded onto a SP Sepharose column (Cytiva) equilibrated in buffer (50 mM HEPES pH 7.4, 250 mM NaCl, 0.5 mM TCEP, 5% glycerol, 5 mM MgCl2, 20 µM GDP)	1h
6	The protein was eluted using a 250 mM to 2.5 M NaCl gradient. Fractions containing LRRK1(FL) were pooled, diluted to ~500 mM NaCl, and incubated with TEV protease overnight at 4°C.	18h
7	The protein was concentrated and put directly over a S200 size exclusion column (Cytiva) equilibrated in storage buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 0.5 mM TCEP, 5% glycerol, 5 mM MgCl2 and 20 µM GDP).	5h
8	The protein was concentrated to ~5-6 µM and flash frozen in liquid nitrogen for storage. N-terminally tagged His6-Z-TEV-LRRK120-2015 was purified using the same protocol.	1h

Protocol references

https://www.biorxiv.org/content/10.1101/2022.11.22.517582v2