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Expression and purification of LRRK2 constructs

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Verena Dederer[1,2], Deep Chatterjee[1,2], Stefan Knapp[1,2], Sebastian Mathea[1,2]

[1]Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, Max-von-Laue-Straße 9, Frankfurt 60438, Germany; [2]Structural Genomics Consortium, Buchman Institute for Molecular Life Science (BMLS), Max-von-Laue-Straße 15, Frankfurt 60438, Germany

Verena Dederer
Goethe University Frankfurt

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

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Abstract

Expression and purification of human LRRK2 and its variants from insect cells.

Guidelines

if not otherwise mentioned every step was performed on ice.

Materials

Equipment

Centrifuge: Thermo Scientific Sorvall LYNX 6000
AKTA: ÄKTA start; ÄKTA Pure 25
Ni-NTA (Cytiva #17531803)
SP-sepharose column (Cytiva #17505701)
HiLoad 16/600 Superdex 200 pg gel filtration column
Amicon Ultra 15 mL Centrifugal Filters 10,000 MWCO (Millipore)

Buffers

Lysis buffer: 50 mM HEPES 7.4 , 500 mM NaCl, 20 mM imidazole, 5 mM $MgCl_2$, 20 µM GDP, 0.5 mM TCEP, 5% glycerol
Ni-NTA elution buffer: 50 mM HEPES 7.4, 500 mM NaCl, 300 mM imidazole, 5 mM $MgCl_2$, 20 µM GDP, 0.5 mM TCEP, 5% glycerol
no salt buffer: 20 mM HEPES 7.4, 5 mM $MgCl_2$, 20 µM GDP, 0.5 mM TCEP, 5% glycerol
high salt buffer: 20 mM HEPES 7.4, 2.5 M NaCl, 5 mM $MgCl_2$, 20 µM GDP, 0.5 mM TCEP, 5% glycerol
gel filtration buffer: 20 mM HEPES 7.4, 150 mM NaCl, 5 mM $MgCl_2$, 20 µM GDP, 0.5 mM TCEP, 0.5% glycerol

Safety warnings

All experiments were performed under the rules of S1 lab regulations.

Before start

Buffers, beads, columns are chillled at 4°C prior to use.

Protein expression and purification

2d 18h

1 exponentially growing SF9 cells (2x10^6 cells/mL in Lonza Insect-XPRESS medium) are transduced with high-titre bacculovirus suspension (encoding TEV cleavable N-terminally His6-Z-tagged human LRRK2).

2 After shaking with 90 rpm at 27 °C for 66:00:00, cells are harvested by centrifugation. 2d 18h

3 Pellets are washed once with PBS and frozen until further use.

4 Cell pellets are resuspended in lysis buffer and lysed by sonication on ice using a 13-mm probe (35% amplitude, 5 sec pulse, 10 sec pause, 5 min total pulse time).

5 Lysate is cleared by ultracentrifugation for 1 hour with 100.000 x g, 4°C

6 Clarified lysate is loaded onto pre-equilibrated Ni-NTA in gravity flow columns.

7 After extensive washing with lysis buffer (20 CV), the His-tagged protein is eluted with elution buffer containing [M] 0.300 Molarity (M) Imidazole

8 After dilution of the eluate's salt concentration to [M] 250 millimolar (mM) NaCl using no salt buffer, the protein is loaded onto a SP sepharose column connected to an Äkta Start system (Cytiva).

9 After washing with buffer containing [M] 250 millimolar (mM) NaCl the bound LRRK2 construct is eluted with a salt gradient ranging from 250 mM to 2.5 M NaCl while elution fractions are collected throughout the run.

10 Peak fractions are pooled and TEV protease (molar ratio of 1:100) is added during overnight incubation rolling at 4 °C .

11 Cleaved tag, TEV protease and other contaminating proteins are removed by an combined SP sephareose-Ni-NTA rebinding.

12 LRRK2 construct is concentrated and subjected to gel filtration in gel filtration buffer on a HiLoad 16/600 S200 column using an Äkta Pure system.

13 Peak fractions are combined, the protein is concentrated.

14 the UV absorbance is measure and the protein is flash frozen in liquid nitrogen for storage.