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Expression and purification of LRRK2 constructs

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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 MgCl₂, 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 MgCl₂, 20 μM GDP, 0.5 mM TCEP, 5% glycerol

no salt buffer: 20 mM HEPES 7.4, 5 mM MgCl₂, 20 μM GDP, 0.5 mM TCEP, 5% glycerol

high salt buffer: 20 mM HEPES 7.4, 2.5 M NaCl, 5 mM MgCl₂, 20 μM GDP, 0.5 mM TCEP, 5% glycerol

gel filtration buffer: 20 mM HEPES 7.4, 150 mM NaCl, 5 mM MgCl₂, 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 chilled at 4°C prior to use.



Protein expression and purification

2d 18h

- 1 exponentially growing SF9 cells (2×10^6 cells/mL in Lonza Insect-XPRESS medium) are transduced with high-titre baculovirus 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  0.300 Molarity (M) Imidazole
- 8 After dilution of the eluate's salt concentration to  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  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.