Aug 15, 2022

Insect cell expression of wildtype and variant LRRK1full length protein

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dx.doi.org/10.17504/protocols.io.rm7vzy6z2lx1/v1

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ABSTRACT

The production of LRRK1 protein and its variants was performed using bacculovirus expression system. The method includes three major steps cloning, large scale expression and protein purification. The high quality LRRK1 protein produced through this method has been used for further biochemical and biophysical studies.

DOI

dx.doi.org/10.17504/protocols.io.rm7vzy6z2lx1/v1

PROTOCOL CITATION

Deep Chatterjee, Sebastian Mathea, Stefan Knapp 2022. Insect cell expression of wildtype and variant LRRK1full length protein . **protocols.io** https://protocols.io/view/insect-cell-expression-of-wildtype-and-variant-lrr-b7ternje

KEYWORDS

LRRK1, Bacculovirus, Protein production

CREATED

Apr 19, 2022

LAST MODIFIED

Aug 15, 2022

PROTOCOL INTEGER ID

60998

GUIDELINES

Expression

Protein quality depends on several factors, for eg. condition of insect cells, virus quality, expression time etc.

Purification

Every steps are performed at 4 °C

Column and beads has to properly equilibrated or regenerated before starting

Protein stability during purification can be improved by using different strategies, such as increasing salt concentration, use of additives or protease inhibitors

MATERIALS TEXT

PURIFICATION BUFFERS

Ni-NTA

Lysis or Wash Buffer

50 mM HEPES pH 7.4, 500 mM NaCl, 20 mM imidazole, 0.5 mM TCEP, 5% glycerol

Elution Buffer

50 mM HEPES pH 7.4 500 mM NaCl, 300 mM imidazole, 0.5 mM TCEP, 5% glycerol

SP chromatography

Buffer Minus

20 mM HEPES pH 7.4 0.5 mM TCEP, 5% glycerol

Buffer Plus

20 mM HEPES pH 7.4, 2.5 M NaCl, 0.5 mM TCEP, 5% glycerol

Buffer 250

20 mM HEPES pH 7.4, 250 mM NaCl, 0.5 mM TCEP, 5% glycerol

GF or Storage Buffer

20 mM HEPES pH 7.4, 150 mM NaCl, 0.5 mM TCEP, 0.5% glycerol

EQUIPMENTS

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Shaker
Infors HT multitron

Centrifuge
Thermo Scientific Sorvall LYNX 6000

AKTA
ÄKTA Basic
ÄKTA Pure 25

MATERIALS

Expression
Insect-XPRESS Medium (Lonza)
3 L-Erlenmeyer glass flask

Purification
Ni-beads (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)

SAFETY WARNINGS

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

Cloning & mutagenesis

1 1h 4m

Amplify the DNA coding for the human LRRK1 residues 20 to 2015 (OHu72031 from Genscript) using the forward primer TACTTCCAATCCGCTGTGTGTCCAGAACGTGCCATGG and the reverse primer TATCCACCTTTACTGTCACCTTCTCTTGCGAGTGCAAGCCTCC. PCR was performed by applying a touch-down protocol.

Thermal cycling procedure:

95 °C **00:10:00**
(**95 °C** **00:00:30** **68 °C** **00:00:30** **68 °C** **00:10:00**) X 5
(**95 °C** **00:00:30** **60 °C** **00:00:30** **68 °C** **00:10:00**) X 5
(**95 °C** **00:00:30** **55 °C** **00:00:30** **68 °C** **00:10:00**) X 5
(**95 °C** **00:00:30** **50 °C** **00:00:30** **68 °C** **00:10:00**) X 20
68 °C **00:10:00**

2

The T4 polymerase-treated amplicon was inserted into the transfer vector pFB-6HZB (SGC) by

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ligation-independent cloning.

3 Point mutations were introduced applying the QuikChange method.

4 The resulting plasmids were utilized for the generation of recombinant Baculoviruses according to the Bac-to-Bac expression system protocol (Invitrogen).

Expression

5 **9.6 L** of exponentially growing Sf9 cells (2 X 10^6 cells/mL in Lonza Insect-XPRESS medium) were infected with high-titre Baculovirus suspension.

6 After **66:00:00** of incubation (**27 °C** and **90 rpm**), cells were harvested by centrifugation. 2d 18h

Purification 1h

7 The pelletes were washed with PBS, re-suspended in lysis buffer (50 mM HEPES pH **7.4** , 500 mM NaCl, 20 mM imidazole, 0.5 mM TCEP, 5% (by vol) glycerol).

8 Lysed by sonication on ice using a 13-mm probe (35% amplitude, 5 s pulse / 10 s pause, 3 min total pulse time).

9 The lysate was cleared by centrifugation (**01:00:00** , **100.000 x g** , **4 °C**). 1h

10 The supernatant is loaded onto a previously equilibrated Ni-beads (Cytiva #17531803) in gravity flow columns.

11 After vigorous washing (20-30 CV) with lysis buffer, the His6-Z tagged protein was eluted in lysis buffer containing imidazole (50 mM HEPES pH **7.4** 500 mM NaCl, 300 mM imidazole, 0.5 mM TCEP, 5% glycerol).

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12 The eluate was diluted with buffer containing no NaCl, (20 mM HEPES pH **7.4** 0.5 mM TCEP, 5% glycerol) in order to reduce the NaCl-concentration to 250 mM

13 The diluted protein was loaded onto an 5 mL SP-Sepharose column (Cytiva #17505701) connected to an ÄKTA Basic system.

14 Wash with buffer containing 250 mM NaCl (20 mM HEPES pH **7.4** , 250 mM NaCl, 0.5 mM TCEP, 5% glycerol) until the absorbance of the flowthrough is below 5 mAU

15 His6 Z TEV-LRRK1 was eluted with a 250 mM to 2.5 M NaCl gradient.

16 The protein was treated with TEV protease (molar ratio TEV to substrate about 1:50) overnight to cleave the His6 Z tag.

17 Contaminating proteins, the cleaved tag, uncleaved protein and TEV protease were removed by another combined SP-Sepharose Ni NTA step.

18 LRRK1 was concentrated and subjected to gel filtration in storage buffer (20 mM HEPES pH **7.4** , 150 mM NaCl, 0.5 mM TCEP, 0.5% glycerol) using an AKTA Pure system combined with an S200 gel filtration column.

19 The final yield as calculated from UV absorbance was 0.1 mg/L.

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