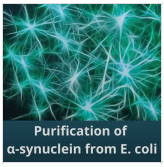

Apr 07, 2021

Purification of α-synuclein from E. coli

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1 Works for me dx.doi.org/10.17504/protocols.io.btynnpve

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ABSTRACT

This protocol details methods for the purification of α-synuclein from E. coli – adapted from Volpicelly-Daley, Nat Protoc 2014.

DOI

dx.doi.org/10.17504/protocols.io.btynnpve

PROTOCOL CITATION

Felix Kraus, vtrinkaus , Ruben Fernandez Busnadiego 2021. Purification of α-synuclein from E. coli. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btynnpve

KEYWORDS

α-synuclein, purification

CREATED

Apr 05, 2021

LAST MODIFIED

Apr 07, 2021

OWNERSHIP HISTORY

Apr 05, 2021 Liz Brydon Protocols.io

Apr 07, 2021 Felix Kraus

PROTOCOL INTEGER ID

48878

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Purification of α-synuclein from E. coli 7h 55m

1 Transform plasmid pT7-7 α-synuclein (α-syn, WT or A53T mutant) into BL21(DE3) cells (Agilent).

pT7-7 plasmids contain an Ampicillin (Amp) resistance.
We thank Hilal Lashuel for depositing the plasmids on Addgene (Addgene, #105727 and #36046).

2

Grow an **Overnight** culture (ONC) of transformed BL21(DE3) in lysogeny broth (LB) medium supplemented with **100 microgram per milliliter (µg/mL) Ampicillin** at **37 °C** .

3

Add **15 mL overnight culture (ONC)** to **1 L terrific broth (TB) medium** , containing **100 microgram per milliliter (µg/mL) Ampicillin** .

4

Incubate at **37 °C** until an OD_{600} of 0.8 to 1.0 is reached.

5h

5 Induce protein expression with **1 Milimolar (mM) IPTG** for **05:00:00** at **37 °C** (see Figure 1).

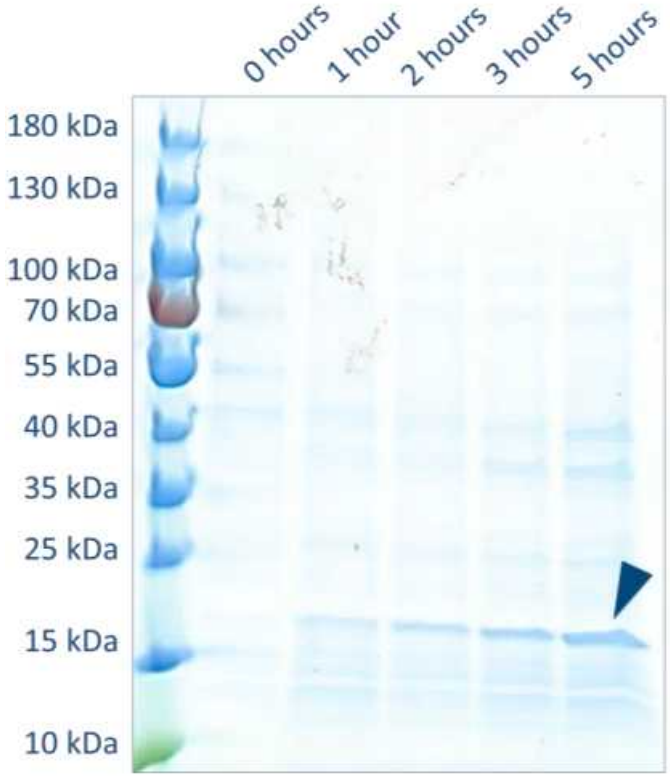

Figure 1: SDS-PAGE of bacterial lysate after induction of protein expression with 1 mM IPTG at 37 °C at different induction times (0,1,2,3,5 h). Note the protein band at 16 kDa (blue arrow head), increasing with time. This band is α-syn.

30m

6

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After 5 h of induction, spin the bacteria at **4000 x g, 00:30:00** and freeze the resulting pellet at **-20 °C** .

7 On the next day, thaw bacterial pellet **On ice** .

8 Resuspend the bacterial pellet in high salt buffer containing **[M]750 Milimolar (mM) NaCl** , **[M]10 Milimolar (mM) Tris, pH 7.6** , **[M]1 Milimolar (mM) EDTA** , and protease inhibitor tablet (cOmplete, Roche - use 50 ml high salt buffer for 1 l of bacterial culture).

9 Sonicate the bacterial suspension with a probe tip of at least 0.25 inch at 60 % power for **00:05:00 (30 sec pulse on, 30 sec pulse off)** at **4 °C** . 5m

10 Boil the sonicated bacterial suspension for **00:15:00** . 15m

We use a glass beaker with boiling water. To avoid spilling of hot bacterial suspension, transfer suspension after sonication into 50 ml reaction tubes that contain holes in the lid, put those reaction tubes into the boiling water. Holes can be created with knifes or scissors.

11 After proteins precipitate during the boiling step (this takes roughly 15 to 20 min), cool the suspension **On ice** .

12 20m

As soon as bacterial suspension is cooled, spin it at **6000 x g, 00:20:00** (make sure that all solid material is in the sediment and the supernatant is clear).

13 15m

Take the supernatant and dialyze it **Overnight** in **[M]10 Milimolar (mM) Tris, pH 7.6** , **[M]50 Milimolar (mM) NaCl** , **[M]1 Milimolar (mM) EDTA** .

Make sure to use a sufficiently large volume for dialysis – for 200 ml protein solution, we use 5 l of dialysis buffer.

14 The next day, concentrate the dialyzed protein with a 3.5 kDa MWCO Amicon Filter device until the desired volume is reached (max. 5 ml for a 16/60 Superdex column, max. 12 ml for a 26/60 column).

15 Filter the protein solution through a syringe filter (0.22 µm). This avoids the loading of aggregated material onto the

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column. Load the filtered solution onto a Superdex 200 column (we recommend using a 26/60 column to allow the loading of larger volumes) and run it with the size exclusion buffer ([M]**10 Milimolar (mM) Tris, pH 7.6** , [M]**50 Milimolar (mM) NaCl** , [M]**1 Milimolar (mM) EDTA**).

16 Start collecting 1.5 ml fractions as soon as the void volume of the column flowed through. α-Syn typically behaves like a 50 kDa protein (collecting 96 fractions for a 26/60 column is usually sufficient).

17 Run a gel of every third fraction collected. Stain and destain, and identify fractions containing α-syn (we use precast gradient gels from Biorad, with a gradient ranging from 4 – 20 % to resolve small proteins) (Figure 2).

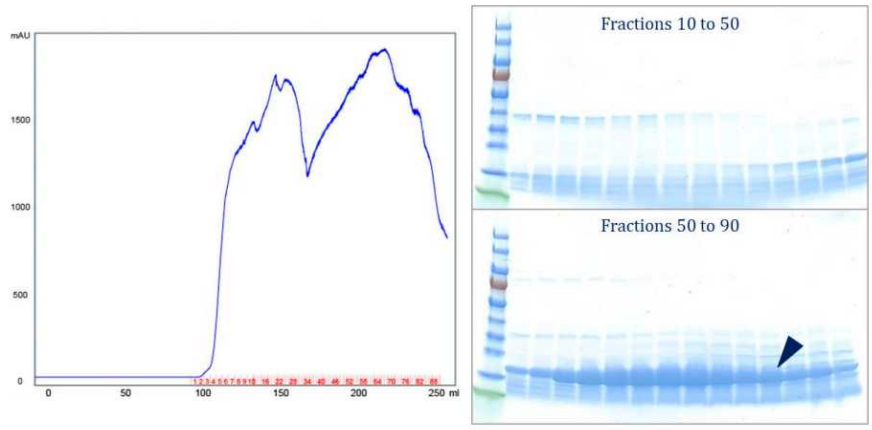

Figure 2: Left: Chromatogram of the dialyzed protein solution on a 26/60 Superdex 200 column. Right: corresponding SDS-PAGE gels of every third fraction eluted from the column. Note, that α-syn elutes over many fractions (blue arrow head), resulting in a large volume of pooled fractions - the α-syn protein band appears at 16 kDa.

18 Pool α-syn containing fractions.

19 15m

Dialyze pooled fraction **Overnight** in [M]**10 Milimolar (mM) Tris, pH 7.6** , [M]**25 Milimolar (mM) NaCl** , [M]**1 Milimolar (mM) EDTA** .

20 The next day, load protein solution onto a cation exchange column (e.g. a MonoQ, make sure that the NaCl concentration in the MonoQ washing buffer is low, so that α-syn can bind nicely to the column matrix).

21 Run a linear gradient ranging from 25 mM NaCl to 1 M NaCl (as described by VolpicellyDaley, Nat Protoc 2014.) on the MonoQ.

We found that one can omit the part of the gradient from 25 mM to 100 mM, and wash instead with 100 mM for one column volume. Additionally, the part of the gradient ranging from 600 mM to 1 M can be omitted as well. However, make sure to wash the column properly in the end to elute all proteins potentially bound to the column.

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22 Collect **1 mL fractions** from 100 mM NaCl to 600 mM NaCl. α-Syn will elute at 300 mM NaCl (Figure 3).

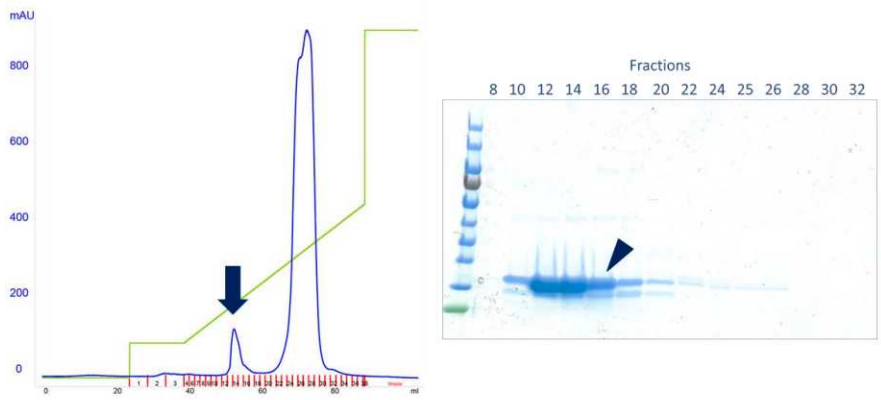

Figure 3: Left: Chromatogram of dialyzed protein solution on an 8 mL MonoQ cation exchange column, Note the symmetrical peak at fraction 14 (blue arrow). Right: SDS-PAGE of every second fraction collected. Note the 16 kDa protein band in fractions 10 to 18, which is α-syn (blue arrow head).

23 Run a gel of all fractions, stain and destain, identify fractions containing α-syn.

24 15m

Pool the fractions containing α-syn and dialyze **Overnight** in **[M]50 Milimolar (mM) Tris, pH 7.5** , **[M]150 Milimolar (mM) KCl** .

25

On the next day, concentrate the protein solution to a final concentration of **[M]5 mg/ml** (330 mM) and freeze it subsequently in batches of 1 ml at **-80 °C** .

26 For conversion of α-syn into fibrils:

26.1 Thaw a tube with 1 ml α-syn monomer of a concentration of 5 mg/ml and centrifuge the solution at **100000 x g, 01:00:00** (to pellet aggregates that might be present), take the supernatant and transfer it to a new 1 ml tube. 1h

26.2 Shake at **900 rpm, 37°C, 24:00:00** in thermomixer.

26.3 Divide the fibrils into **50 µl aliquots** .

We use PCR tubes; Fibrils can be stored at **-80 °C** .

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