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a-Synuclein protein expression and purification

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Protocol status: Working

We use this protocol and it's working

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rt-quic

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Abstract

Protocol for recombinant a-synuclein purification, useful as monomer template for seeded-amplification assays (like RT_QUIC or PMCA). Recommendations are to store the protein always on ice while not running and do not stop purification when it is started.

Materials

- 1. BL21-CodonPlus (DE3)-RIL Chemical Competent Cells (Agilent #230245-41)
- 2. Thermo Scientific™ Low Protein Binding Collection Tubes (1.5 mL) PI90411
 - X Nalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units Thermo Fisher Scientific Catalog #565-0010
 - 🔀 SnakeSkin Dialysis Tubing 3.5K MWCO 35 Thermo Fisher Scientific Catalog #88244
 - X Endotoxin detection kit LAL Genscript Catalog #95045-024
 - **⊠** ToxinEraser[™] Endotoxin Removal Kit **Genscript Catalog** #89233-330
 - **⊠** ToxinEraser™ Endotoxin Removal Resin **Genscript Catalog** #L00402
 - 🔀 HiPrep Q HP anion exchange chromatography column Cytiva Catalog #29018182
 - MilliporeSigma™ Amicon™ Ultra-15 Centrifugal Filter Units Catalog #MilliporeSigma™ UFC901024

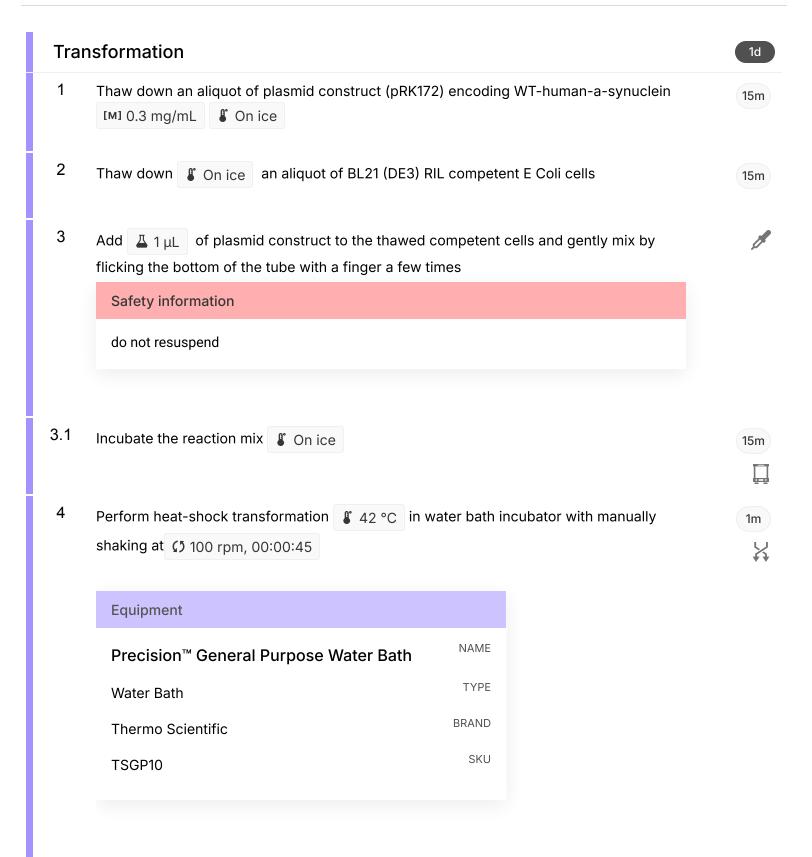


Protocol materials

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Troubleshooting







5 Immediately transfer the tube on ice and incubate for 1 min.

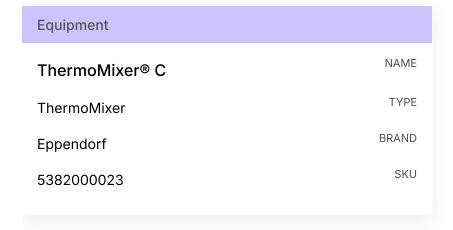
1m

6 Add \perp 1000 μ L of SOC media to a chilled reaction

10s

7 Incubate the bacteria (5) 200 rpm, 37°C, 00:30:00

30m



- 7.1 Prepare sterile 10cm LB agar plate containing [M] 0.1 mg/mL of ampicillin
- 8 Centrifuge at \$\infty\$ 500 x g, 10°C, 00:03:00 and discard the supernatant leaving

3m

- Δ 50 μL of media
- 8.1 Spread 50 ul of cell suspension onto a selection plate and incubate overnight at

10m

§ 37 °C in bacterial incubator





Equipment Isotemp™ Microbiological Incubator, 178 L, Stainless Steel NAME TYPE Microbiological Incubator BRAND Fisherbrand SKU 15-103-0513

Protein expression

12h

9 Pick one colony and transfer into ∠ 10 mL LB media with [M] 0.1 mg/mL of ampicillin start in the morning (9:00 am)

9.1 Incubate the bacteria 250 rpm, 37°C, 05:00:00 until it reaches OD 0.2-0.3

5h

Equipment NAME Natural convection incubator **TYPE** Bacterial shaker **BRAND** Innova SKU M1335-0000



Transfer a starter culture to 2X2L flasks filled with 0.5L LB media with IMI 0.1 mg/mL of ampicillin 5 mL to each flask

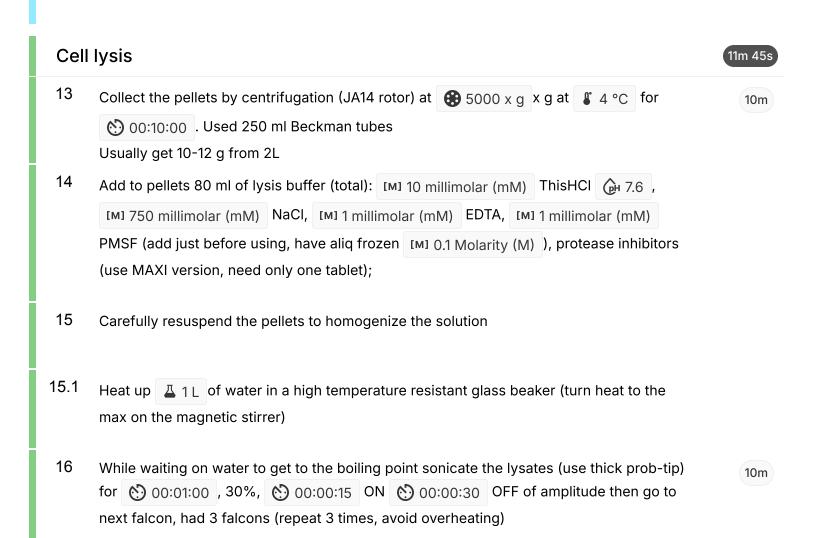
Incubate the culture at the same conditions until it reaches OD 0.8 (use nanodrop or cuvette) (reaches optimal density at 6-7 pm)

Induce protein expression by adding IMI 0.05 millimolar (mM) IPTG, incubate at 12h

Note

Note

To cool down the grown culture, transfer the flasks into ice-bath and incubate until it reaches desired temperature



sure it does not leak.

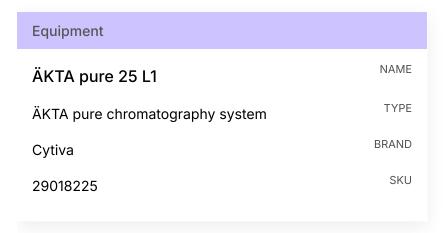


17	After sonication samples need to get boiled thereby put the falcon tubes into glass beaker and boil for 00:25:00. Use tweezers to pull out the tubes	25m
18	Transfer boiled homogenates into new 50 mL falcon tubes; chill down suspensions at room temperature for 20 min	20m
19	Prepare 4 L of buffer [M] 10 millimolar (mM) TrisHCl 🖟 7.6,	
	[м] 50 millimolar (mM) NaCl, [м] 1 millimolar (mM) EDTA, [м] 1 millimolar (mM)	
	PMSF for dialysis	
20	Centrifuge the homogenates at 20000 x g for 01:00:00 at 4 °C	1h
21	Filter the supernatant using 0.45 um filter unit	
	Nalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units Thermo Fisher Scientific Catalog #565-0010	
22	Transfer filtered supernatant into dialysis bag which is: SnakeSkin Dialysis tubing, 3.5K MWCO, 35 mm dry I.D., 35 feet. Measure the dialysis tube taking into consideration that 5 cm length of tube holds 48 mL of the sample (plus 2.5cm at each end for closure). Clip the tube using green clips, make	~

Place the dialysis bag into 🚨 4 L plastic beaker filled with dialysis buffer, incubate overnight on magnetic plate on the slow mode (Chromatography fridge)

SnakeSkin Dialysis Tubing 3.5K MWCO 35 Thermo Fisher Scientific Catalog #88244





Protein purification (anion-exchange chromatography)

- After a night of dialysis (4 °C slow mixing) collect the suspension into 100 mL glass bottle (filter the sample before running on the column, 0.22 um filter).
- 24.1 Wash the column 2V of miliQ degassed water
- Wash the column with 2V of **STARTING BUFFER** [M] 10 millimolar (mM) TrisHCl PH 7.6, [M] 50 millimolar (mM) NaCl
- 24.3 Activate with 1V of [M] 10 millimolar (mM) TrisHCl (pH 7.6 , [M] 1 Molarity (M) NaCl
- 24.4 Equilibrate with 3V of starting buffer
- 25 Load 4 80 mL of suspension and then washed with 100 ml [M] 50 millimolar (mM)



NaCl [M] 10 millimolar (mM) TrisHCL, 4 300 mL of gradient elution (0-100%), 2 ml/min flow rate. Collected samples using fraction collector 2, every fraction 4 ml (use 10 ml glass tubes)

- Place supernatant into channel A1 (was previously use for starting buffer, do not generate bubbles)
- 27 Place starting buffer in channel A2 (clean the tubing using the program mode)
- Place elution buffer in channel B1 ([M] 10 millimolar (mM) TrisHCl 7.6 ,

 [M] 1 Molarity (M) NaCl)

 Collected samples using fraction collector 2, every fraction 4 ml (use 10 ml glass tubes);
- Analyze the fractions eluted at 250-350 mM salt (20 RFU conductivity) though SDS-PAGE (stain with Coomassie).

- Measure A280/260 for the fractions containing single a-syn band, avoid collecting samples with A280/260 > 0.85
- 31 Combine the evaluated factions and measure total protein concentration using nanodrop.
- Dialyze with 4 L of [M] 10 millimolar (mM) TrisHCl 4 7.6, NaCl (follow instruction for dialysis)

T

Further purification

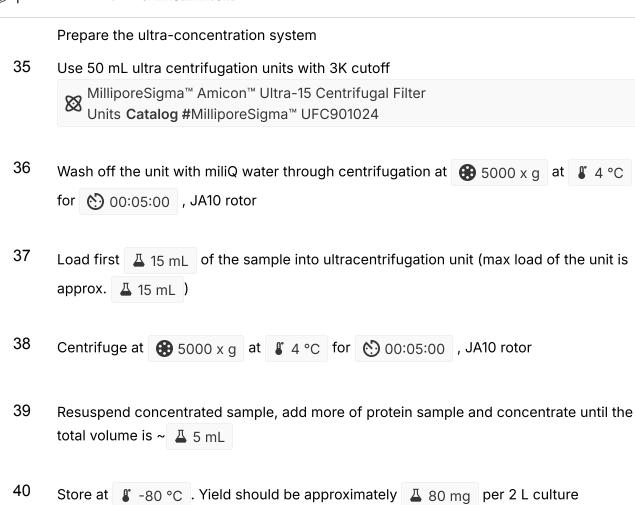
Repeat section 'Protein purification (anion-exchange chromatography)' for the further fractionation of the purified preparation by go to step #23



Protein concentration



Concentrate dialyzed protein sample to approximately [M] 30 mg/mL aliquot



Endotoxin removal

Follow instructions for
 ★ ToxinEraser™ Endotoxin Removal Kit Genscript Catalog #89233-330 with modifications
 For a more successful endotoxin removal, add 1 mL of
 ★ ToxinEraser™ Endotoxin Removal Resin Genscript Catalog #L00402 before the regeneration process
 Collect the eluate into 5 mL endotoxin-free tube and save 2 aliquots (10 μL and 50 μL) for protein concentration and endotoxin measurements

Endotoxin quantification

5m

5m



43 Follow instructions for Endotoxin detection kit LAL Genscript

🔀 Endotoxin detection kit LAL Genscript Catalog #95045-024