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Human and mouse alpha-synuclein protein expression and purification



Forked from a-Synuclein protein expression and purification



Science Advances



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We use this protocol and it's working well

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Abstract

The protocol for is designed for high-yield purification of recombinant α -synuclein monomer. It is recommended to always store the protein on ice, and once the purification process has started, it should not be stopped.



Materials

- BL21-Gold (DE3) Competent Cells Agilent Technologies Catalog #230132
- 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
- Endotoxin detection kit LAL Genscript Catalog #95045-024
- X ToxinEraser™ Endotoxin Removal Kit Genscript Catalog #89233-330
- X 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

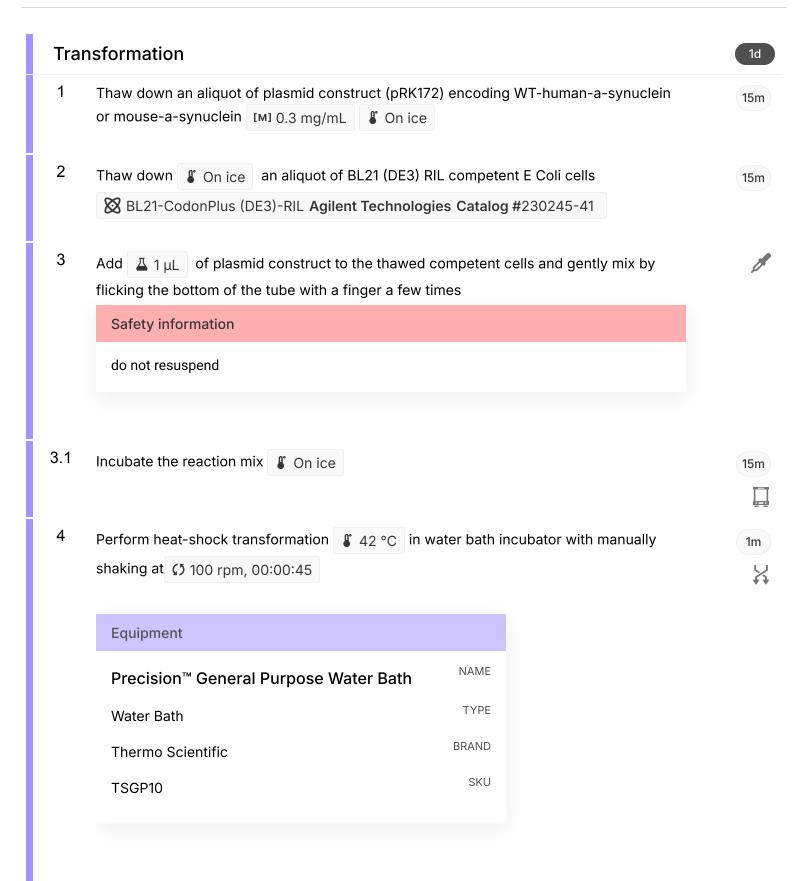


Protocol materials

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- X ToxinEraser™ Endotoxin Removal Resin Genscript Catalog #L00402
- Endotoxin detection kit LAL Genscript Catalog #95045-024
- BL21-CodonPlus (DE3)-RIL Agilent Technologies Catalog #230245-41
- 🔯 1.5mL Micro Centrifuge Tube; endotoxin-free CELLTREAT Catalog #50-202-024
- 🏻 ToxinEraser™ Endotoxin Removal Kit **Genscript Catalog #**89233-330
- X ToxinEraser™ Endotoxin Removal Resin **Genscript Catalog** #L00402
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- X SnakeSkin Dialysis Tubing 3.5K MWCO 35 Thermo Fisher Scientific Catalog #88244
- Endotoxin detection kit LAL Genscript Catalog #95045-024
- 🔯 SOC Medium Merck MilliporeSigma (Sigma-Aldrich) Catalog #S1797-10X5ML
- 🔯 Ampicillin sodium salt Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0166
- 🔯 Agar powder **Grainger Catalog #**31FZ34
- 🔯 LB Broth, Miller (Granulated) **Fisher Scientific Catalog #**BP9723-500
- X IPTG Research Products International Corp (RPI) Catalog #I56000-5.0
- SODIUM CHLORIDE Fisher Scientific Catalog #S2711
- EDTA, disodium salt, dihydrate **Fisher Scientific Catalog** #S312-500
- cOmplete™, EDTA-free Protease Inhibitor Cocktail MIDI Merck MilliporeSigma (Sigma-**Aldrich) Catalog #4693132001**
- Tris Base Research Products International Corp (RPI) Catalog #T60040-5000.0

Troubleshooting







5	Immediately	/ transfer	the tube	on ice and	incubate	for 1 r	min.
_	miniculation	,	tile tube		IIICUDUIC	101 11	111111

1m

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

10s

SOC Medium Merck MilliporeSigma (Sigma-Aldrich) Catalog #S1797-10X5ML

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

30m

ThermoMixer® C ThermoMixer ThermoMixer Eppendorf S382000023 ThermoMixer SKU

7.1 Prepare sterile 10cm LB agar plate containing [M] 0.1 mg/mL of ampicillin

X Ampicillin sodium salt Merck MilliporeSigma (Sigma-Aldrich) Catalog #A0166

🔀 Agar powder **Grainger Catalog #**31FZ34

8 Collect 50 ul of cell suspension (Tube #1 5% of cells)

Centrifuge 950 cell suspension at \$\begin{align*} \text{500 x g, 10°C, 00:03:00} \end{align*}

Save the pellet with approximately \perp 50 μ of media (Tube #2 95% of cells)

8.1 Spread tubes #1 and #2 onto a selection plate and incubate overnight at 37 °C in

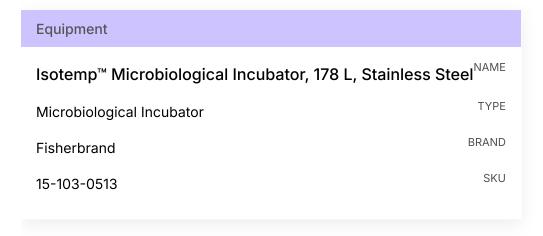
bacterial incubator

10m

3m

1





Protein expression

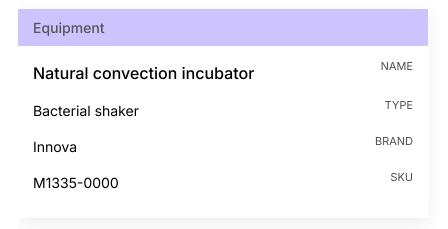
12h

9 Pick one colony and transfer into 4 10 mL LB media with [M] 0.1 mg/mL of ampicillin start in the morning (9:00 am)

☑ LB Broth, Miller (Granulated) Fisher Scientific Catalog #BP9723-500

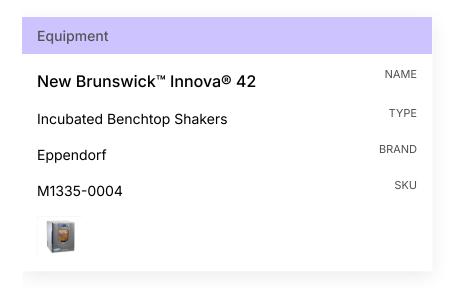
9.1 Incubate the bacteria (5) 250 rpm, 37°C, 05:00:00 until it reaches OD 0.2-0.3

5h

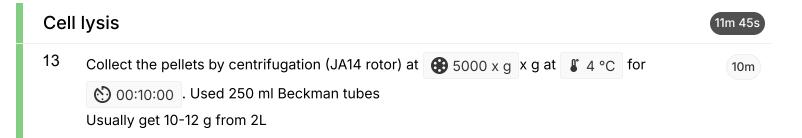




10 Transfer a starter culture to 2X2L flasks filled with 0.5L LB media with [M] 0.1 mg/mL of ampicillin 5 mL to each flask



11 Incubate the culture at the same conditions until it reaches OD 0.8 (use nanodrop or 5h cuvette) (reaches optimal density at 6-7 pm) 12 Induce protein expression by adding [M] 0.05 millimolar (mM) IPTG, incubate at 12h \$ 18 °C for (*) 12:00:00 overnight IPTG Research Products International Corp (RPI) Catalog #I56000-5.0 Note To cool down the grown culture, transfer the flasks into ice-bath and incubate until it reaches desired temperature







- Add to pellets 80 ml of lysis buffer (total): [M] 10 millimolar (mM) ThisHCl → 7.6, [M] 750 millimolar (mM) NaCl, [M] 1 millimolar (mM) EDTA, [M] 1 millimolar (mM) PMSF (add just before using, have aliq frozen [M] 0.1 Molarity (M)), protease inhibitors (use MAXI version, need only one tablet);

 SODIUM CHLORIDE Fisher Scientific Catalog #S2711

 Tris Base Research Products International Corp (RPI) Catalog #T60040-5000.0

 EDTA, disodium salt, dihydrate Fisher Scientific Catalog #S312-500

 COmplete™, EDTA-free Protease Inhibitor Cocktail MIDI Merck MilliporeSigma (Sigma-Aldrich) Catalog #4693132001
- 15 Carefully resuspend the pellets to homogenize the solution
- Heat up L 1L of water in a high temperature resistant glass beaker (turn heat to the max on the magnetic stirrer)
- While waiting on water to get to the boiling point sonicate the lysates (use thick prob-tip) for 00:01:00, 30%, 00:00:15 ON 00:00:30 OFF of amplitude then go to next falcon, had 3 falcons (repeat 3 times, avoid overheating)

10m



17 After sonication samples need to get boiled thereby put the falcon tubes into glass 25m beaker and boil for 00:25:00. Use tweezers to pull out the tubes 18 Transfer boiled homogenates into new 50 mL falcon tubes; chill down suspensions at 20m room temperature for 20 min 19 Prepare

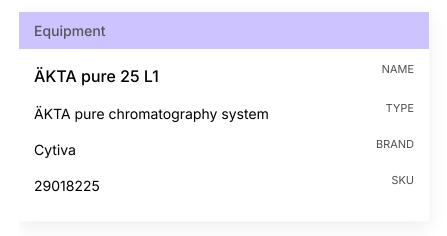
4 L of buffer [M] 10 millimolar (mM) TrisHCl (pH 7.6 , [M] 50 millimolar (mM) NaCl, [M] 1 millimolar (mM) EDTA, [M] 1 millimolar (mM) PMSF for dialysis 20 Centrifuge the homogenates at 3 20000 x q for 0 01:00:00 at 4 4 °C 1h 21 Filter the supernatant using 0.45 um filter unit Nalgene™ Rapid-Flow™ Sterile 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

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 sure it does not leak.

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** [м] 10 millimolar (mM) TrisHCl ф 7.6 , [м] 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)
- Analyze the fractions eluted at 250-350 mM salt (20 RFU conductivity) though SDS-PAGE (stain with Coomassie).

 Combine 10 μL of each fraction with 10 μL of 2X laemmli buffer and analyze fractions by SDS-PAGE with 4–20% gradient gels, followed by coomassie staining/destaining
- 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 [м] 10 millimolar (mM) TrisHCl 🕞 7.6 ,

1

Further purification

Repeat section 'Protein purification (anion-exchange chromatography)' for the further fractionation of the purified preparation



Protein concentration



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



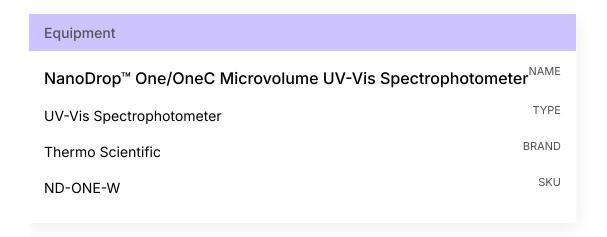
Add \perp 3 μ L of 10x diluted aliquot in PBS onto nanodrop pedestal;

Parameters:

- other proteins; coefficient extinction: 5.98; MW: 14.4 kDA (for wild-type human asynuclein)
- other proteins; coefficient extinction: 7.45; MW: 14.4 kDA (for wild-type mouse asynuclein)

Perform two measurements and confirm <10% standard error between two measurements

If necessary, prepare 20X and 30X dilutions to confirm findings.



Prepare the ultra-concentration system

35 Use 50 mL ultra centrifugation units with 3K cutoff

MilliporeSigma™ Amicon™ Ultra-15 Centrifugal Filter Units **Catalog** #MilliporeSigma™ UFC901024

- Wash off the unit with miliQ water through centrifugation at \$5000 x g at \$4 °C for 00:05:00 , JA10 rotor
- 37 Load first $\stackrel{\bot}{\bot}$ 15 mL of the sample into ultracentrifugation unit (max load of the unit is approx. $\stackrel{\bot}{\bot}$ 15 mL)
- Centrifuge at \$\infty\$ 5000 x g at \$\infty\$ 4 °C for \$\infty\$ 00:05:00 , JA10 rotor

5m

- Resuspend concentrated sample, add more of protein sample and concentrate until the total volume is $\sim 4.5 \, \text{mL}$
- 40 Store at 🖁 -80 °C . Yield should be approximately 🚨 80 mg per 2 L culture

Endotoxin removal

- 41 Follow instructions for
 - **⊠** ToxinEraser[™] Endotoxin Removal Kit **Genscript Catalog** #89233-330 with modifications

For a more successful endotoxin removal, add 4 1 mL of

X ToxinEraser™ Endotoxin Removal Resin **Genscript Catalog** #L00402 before the regeneration process

Collect the eluate into \square 5 mL endotoxin-free tube and save 2 aliquots (\square 10 μ L and \square 50 μ L) for protein concentration and endotoxin measurements

Endotoxin quantification

- 43 Follow instructions for Endotoxin detection kit LAL Genscript