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We use this protocol and it's working well

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## Human and mouse alpha-synuclein protein expression and purification

 Forked from [a-Synuclein protein expression and purification](#)

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### ABSTRACT

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

**Keywords:** ASAPCRN

## MATERIALS

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 BL21-Gold (DE3) Competent Cells **Agilent Technologies Catalog #230132**

 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**

 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

 BL21-Gold (DE3) Competent Cells **Agilent Technologies Catalog #230132** Materials

 IPTG **Research Products International Corp (RPI) Catalog #I56000-5.0** Step 12

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

Step 14

 Nalgene™ Rapid-Flow™ Sterile Single Use Vacuum Filter Units **Thermo Fisher Scientific Catalog #565-0010**

Materials, Step 21

 ToxinEraser™ Endotoxin Removal Resin **Genscript Catalog #L00402**

Materials, Step 41

 1.5mL Micro Centrifuge Tube; endotoxin-free **CELLTREAT Catalog #50-202-024**

Step 42

 EDTA, disodium salt, dihydrate **Fisher Scientific Catalog #S312-500** Step 14

 BL21-CodonPlus (DE3)-RIL **Agilent Technologies Catalog #230245-41** Step 2

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

Step 7.1

 LB Broth, Miller (Granulated) **Fisher Scientific Catalog #BP9723-500** Step 9

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

Materials, Step 22

 SODIUM CHLORIDE **Fisher Scientific Catalog #S2711** Step 14

 Endotoxin detection kit LAL **Genscript Catalog #95045-024** Materials, Step 43

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

Step 6

 Agar powder **Grainger Catalog #31FZ34** Step 7.1

 cComplete™, EDTA-free Protease Inhibitor Cocktail MIDI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4693132001**

Step 14

 HiPrep Q HP anion exchange chromatography column **Cytiva Catalog #29018182**

Materials, Step 24

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

Materials, Step 35

 ToxinEraser™ Endotoxin Removal Kit **Genscript Catalog #89233-330**

Materials, Step 41

## Transformation

1d

- 1 Thaw down an aliquot of plasmid construct (pRK172) encoding WT-human-a-synuclein or mouse-a-synuclein [M] 0.3 mg/mL  On ice

15m

- 2 Thaw down  On ice an aliquot of BL21 (DE3) RIL competent E Coli cells

15m

 BL21-CodonPlus (DE3)-RIL **Agilent Technologies Catalog #230245-41**

- 3 Add  1 µL of plasmid construct to the thawed competent cells and gently mix by flicking the bottom of the tube with a finger a few times



### Safety information

do not resuspend

- 3.1 Incubate the reaction mix 

15m



- 4 Perform heat-shock transformation  42 °C in water bath incubator with manually shaking at

1m

 100 rpm, 00:00:45



### Equipment

#### Precision™ General Purpose Water Bath

NAME

Water Bath

TYPE

Thermo Scientific

BRAND

TSGP10

SKU

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

1m

6 Add 1000 µL of SOC media to a chilled reaction

10s

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

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

30m

### Equipment

ThermoMixer® C

NAME

ThermoMixer

TYPE

Eppendorf

BRAND

5382000023

SKU

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

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)

3m

Centrifuge 950 cell suspension at 500 x g, 10°C, 00:03:00

Save the pellet with approximately 50 µL 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 bacteria 10m incubator



#### Equipment

**Isotemp™ Microbiological Incubator, 178 L, Stainless Steel**<sup>NAME</sup>

Microbiological Incubator TYPE

Fisherbrand BRAND

15-103-0513 SKU

## 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)

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

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

5h

#### Equipment

**Natural convection incubator** NAME

Bacterial shaker TYPE

Innova BRAND

M1335-0000 SKU

- 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

### Equipment

#### New Brunswick™ Innova® 42

Incubated Benchtop Shakers

NAME

Eppendorf

TYPE

M1335-0004

BRAND

SKU



- 11 Incubate the culture at the same conditions until it reaches OD 0.8 (use nanodrop or cuvette) (reaches optimal density at 6-7 pm) 5h

- 12 Induce protein expression by adding [M] 0.05 millimolar (mM) IPTG, incubate at  $\text{18 }^{\circ}\text{C}$  for 12h

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

## Cell lysis

11m 45s

- 13 Collect the pellets by centrifugation (JA14 rotor) at 5000 x g x g at  $\text{4 }^{\circ}\text{C}$  for 00:10:00 . Use 10m  
250 ml Beckman tubes  
Usually get 10-12 g from 2L

## Equipment

**Avanti® J-E Centrifuge**

NAME

Avanti Centrifuge

TYPE

Beckman

BRAND

369005

SKU

**14**

Add to pellets 80 ml of lysis buffer (total): [M] 10 millimolar (mM) TrisHCl pH 7.6, [M] 750 millimolar (mM) NaCl, [M] 1 millimolar (mM) EDTA, [M] 1 millimolar (mM) PMSF (add just before using, have aliquot 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**

cComplete™, EDTA-free Protease Inhibitor Cocktail MIDI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4693132001**

**15**

Carefully resuspend the pellets to homogenize the solution

**15.1**

Heat up 1 L of water in a high temperature resistant glass beaker (turn heat to the max on the magnetic stirrer)

**16**

While waiting on water to get to the boiling point sonicate the lysates (use thick probe-tip) for 30%, 00:00:15 ON 00:00:30 OFF of amplitude then go to next falcon, had 3 falcons (repeat 3

times, avoid overheating)

- 17 After sonication samples need to get boiled thereby put the falcon tubes into glass beaker and boil for 25m  
⌚ 00:25:00 . Use tweezers to pull out the tubes
- 18 Transfer boiled homogenates into new 50 mL falcon tubes; chill down suspensions at room temperature for 20m  
20 min
- 19 Prepare  $\Delta$  4 L of buffer [M] 10 millimolar (mM) TrisHCl  $\text{pH}$  7.6, [M] 50 millimolar (mM) NaCl, [M] 1 millimolar (mM) EDTA, [M] 1 millimolar (mM) PMSF for dialysis
- 20 Centrifuge the homogenates at  $\odot$  20000 x g for ⌚ 01:00:00 at  $\text{fl}^*$  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 sure it does not leak.*  
Place the dialysis bag into  $\Delta$  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

## Equipment

ÄKTA pure 25 L1	NAME
ÄKTA pure chromatography system	TYPE
Cytiva	BRAND
29018225	SKU

## Protein purification (anion-exchange chromatography)

- 23 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 Column - HiPrep Q HP 16/10 column 1x20 ml (stored in 70% ethanol);  
 HiPrep Q HP anion exchange chromatography column **Cytiva Catalog #29018182**
- 24.1 Wash the column 2V of miliQ degassed water
- 24.2 Wash the column with 2V of **STARTING BUFFER** [M] 10 millimolar (mM) TrisHCl  7.6 , [M] 50 millimolar (mM) NaCl
- 24.3 Activate with 1V of [M] 10 millimolar (mM) TrisHCl  7.6 , [M] 1 Molarity (M) NaCl

## 24.4 Equilibrate with 3V of starting buffer

- 25 Load  $\Delta$  80 mL of suspension and then washed with 100 ml [M] 50 millimolar (mM) NaCl [M] 10 millimolar (mM) TrisHCL,  $\Delta$  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)
- 26 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)
- 28 Place elution buffer in channel B1 ([M] 10 millimolar (mM) TrisHCl pH 7.6, [M] 1 Molarity (M) NaCl) Collected samples using fraction collector 2, every fraction 4 ml (use 10 ml glass tubes);
- 29 Analyze the fractions eluted at 250-350 mM salt (20 RFU conductivity) though SDS-PAGE (stain with Coomassie).  
*Combine  $\Delta$  10  $\mu$ L of each fraction with  $\Delta$  10  $\mu$ L of 2X laemmli buffer and analyze fractions by SDS-PAGE with 4–20% gradient gels, followed by coomassie staining/destaining*
- 30 Measure A280/260 for the fractions containing single a-syn band, avoid collecting samples with A280/260 > 0.85
- 31 Combine the evaluated fractions and measure total protein concentration using nanodrop.

32

Dialyze with 4 L of [M] 10 millimolar (mM) TrisHCl (pH 7.6), [M] 50 millimolar (mM) NaCl (follow instruction for dialysis)

33

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

## Protein concentration

10m

34

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

Add 3 µL of 10x diluted aliquot in PBS onto nanodrop pedestal;

Parameters:

- other proteins; coefficient extinction: 5.98; MW: 14.4 kDa (**for wild-type human α-synuclein**)
- other proteins; coefficient extinction: 7.45; MW: 14.4 kDa (**for wild-type mouse α-synuclein**)

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

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

### Equipment

#### NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer<sup>NAME</sup>

UV-Vis Spectrophotometer

TYPE

Thermo Scientific

BRAND

ND-ONE-W

SKU

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

- 36 Wash off the unit with miliQ water through centrifugation at 5000 x g at 4 °C for 00:05:00 , 5m JA10 rotor
- 37 Load first 15 mL of the sample into ultracentrifugation unit (max load of the unit is approx. 15 mL )
- 38 Centrifuge at 5000 x g at 4 °C for 00:05:00 , JA10 rotor 5m
- 39 Resuspend concentrated sample, add more of protein sample and concentrate until the total volume is ~ 5 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 1 mL of ToxinEraser™ Endotoxin Removal Resin **Genscript Catalog #L00402** before the regeneration process
- 42 Collect the eluate into 5 mL endotoxin-free tube and save 2 aliquots ( 10 µL and 50 µL ) for protein concentration and endotoxin measurements  
 1.5mL Micro Centrifuge Tube; endotoxin-free **CELLTREAT Catalog #50-202-024**

## Endotoxin quantification

43 Follow instructions for Endotoxin detection kit LAL Genscript

 Endotoxin detection kit LAL **Genscript Catalog #95045-024**