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Recombinant αS purification Protocol

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details the recombinant αS purification.

Attachments

DOCX

Generation of recomb...

22KB

Materials

Lysis Buffer:

	A	B
	NaOH	40 mM
	Tris pH 8.0	20 mM
	EDTA	1 mM
	Triton X-100	0.10%

Buffer A:

	Thris pH 8.0	25 mM
	NaCl	20 mM
	EDTA	1 mM

Buffer B (filtered) :

	Tris pH 8.0	25 mM
	NaCl	1M
	EDTA	1mM

Buffer C (filtered) :

	Tris pH 8	25mM
	NaCl	1M
	EDTA	1mM

Culture Growth and Induction

1d 14h 5m

1 **Day 1:** Inoculate 5 mL of LB/Amp [100 µL : 1 µL of 100 µL Amp stock (freezer) for every 1 mL of culture] with 1 colony from LB/Amp plate and put in a shaker at 37 °C w/ 250 rpm Overnight .

8h

- Modification:

1. Inoculate 10 mL of LB/Amp with 1 colony (allows more rapid growth).

- If no viable plate:

1. Take a stab from glycerol stock (-80 °C Freezer) and grow Overnight .
2. Streak LB/Amp agar plate with Overnight culture.

2 **Day 2:** Inoculate 500 mL of LB/Amp (100 µL : 1 µL of 100 µL stock for every 1 mL of culture) with 5 mL of Overnight culture and put in shaker at 37 °C until O.D = 0.5 – 0.6 (~1.5h - 03:00:00)

3h

- Modification:

1. Inoculate 1000 mL of LB/Amp with 10 mL of Overnight culture and put in shaker.
2. Nano-drop:

- Use 2 µL drop.
- Use LB or LB/Amp as a blank.

3. Formula to determine the time it will take the culture to hit an OD of 0.5 (assuming 30 min doubling time):

$$OD_f = OD_i \left[2^{(t/30min)}\right]$$

For example: for a culture with an OD$_i$ = 0.052

$$0.5 = 0.052 \left[2^{(t/30min)}\right]$$

$$9.61 = \left[2^{(t/30min)}\right]$$

$$\ln[9.61] = t/30[\ln(2)]$$

t=97.3 minutes

3 **Induce with IPTG (** 400 µL **:** 6.4 µL **of** [M] 0.5 Mass Percent **) for every** 10 mL **of culture) for 4h-** 06:00:00 **at** 37 °C **.** 6h

4 Spin down culture at 4600 x g, 4°C, 00:20:00 in 500 mL buckets (max volume 350 mL) in JLA-10.5 rotor (blue rotor, standing centrifuge). 20m

Note

First Stopping Point: Pellet can be frozen at -20 °C . For > 500 mL culture, split pellet into 500 mL equivalent fractions

5 **Day 3**: Resuspend in 50 mL of lysis buffer. Add 1 tablet of protease inhibitor cocktail (NOT MINI) and 50 µL of 500 µL PMSF (500 µL stock in floor -20 °C ; Final conc. 500 µL ; incubate at 37 °C without shaking for 35min- 00:40:00 . 40m

Lysis Buffer:

	A	B
	NaOH	40 mM
	Tris pH 8.0	20 mM
	EDTA	1 mM

	A	B
	Triton X-100	0.1%

6 Add 500 µL of [M] 1 Mass Percent MgCl2, 500 µL of [M] 1 Mass Percent CaCl2, and 20 µL of DNase from Roche (200 µL total) and incubate at 37 °C with 250 rpm shaking for 01:00:00 hour. 1h

7 Add 1 mL [M] 0.25 Mass Percent EDTA, mix well, and remove cellular debris by centrifugation at 16900 x g, 00:15:00 (JA 25.50 rotor). 15m

8 Add 0.116 g of ammonium sulfate per mL of supernatant and stir at 4 °C for 01:00:00 . Centrifuge at 20000 x g for 00:30:00 , use new tubes. (~ 5.8 g ; Toss pellet). 1h 30m

9 Add 0.244 g of ammonium sulfate per mL of supernatant and stir at 4 °C for 01:00:00 (or Overnight). Centrifuge at 20000 x g for 00:30:00 , use new tubes. (~ 12.9 g ; **Keep pellet). 2h 30m

- Quality Control Checkpoint: Pellet should be at bottom of tube. Excessive proteolysis is indicated by floating pellet or significant smearing of pellet along the side of the tube.

10 **Day 4:** Resolubilize in 25 mL s Buffer A and add PMSF to 1 µL . Stir in refrigerator for ~ 00:30:00 . 30m

Buffer A:

	Thris pH 8.0	25 mM
	NaCl	20 mM
	EDTA	1 mM

11 Dialyze against 1 L of Buffer A with 0.5 µL PMSF Overnight . 8h

12 **Day 5:** Filter through 0.22 µL filter, Milex, Millipore.

13 LPLC: Run over Anion Exchange column HiTrap Q FF (program: SC IExAlphasS, Buffer A, filtered Buffer B), aS will start to come off at ~20% Buffer B). Keep samples at 4 °C.

SEE DETAILED QFF PROTOCOL

Buffer B (filtered):

	A	B
	Tris pH 8.0	25 mM
	NaCl	1 M
	EDTA	1 mM

14 Concentrate αS positive fractions with Amicon Ultracel-3KD MWCO, Millipore, and exchange into filtered Buffer C. Perform during Day 6 equilibration. 20m

- Spin the samples at 4500 x g for ~ 00:20:00 till final sample volume <= 1 mL (lower volume better e.g. 750 µL).

Buffer C (filtered):

	A	B
	Tris pH 8	25 mM
	NaCl	1 M
	EDTA	1 mM

15 Day 6: LPLC: Run over size exclusion column in Buffer C. aS will start to come off at ~ 80 mL . **SEE DETAILED SEC PROTOCOL – Freeze tubes until Conc./BCA.**

16 Concentrate aS positive fractions with Amicon Ultracel-3KD MWCO, Millipore, and exchange into sterile PBS.

- BCA samples and aliquot at desired concentration/volume (recommended at 5 µL).
- Freeze aliquots at -80 °C