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In devel.

ActA purification protocol

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Buffers

1 ***Lysis Buffer:***
20mM MOPS
100mM KCl
5mM Imidazole pH 7

Wash Buffer:
20mM MOPS
100mM KCl
20mM Imidazole pH 7

Elution Buffer:
20mM MOPS
100mM KCl
250mM Imidazole pH 7

Storage Buffer:
20mM HEPES
100 NaCl pH 7.5

Day 1

2 ▪ Spread the bacteria stock from -80 (tray D – fist box tube name “Listeria ActA-His DPL 1545”) in BHI plate (no antibiotic), and incubate @ 37°C for overnight.

3 ▪ Make Brain Heart Infusion media (BHI)

Day 2

4 ▪ Start 25ml culture + 7.14ul chloramphenicol @37°C for overnight

Day 3

5 ▪ Transfer the 25ml starting culture into (475ml BHI+135.7ul chloramphenicol) and grow @37°C for 6-8 hours

6 ▪ Spin down cells in 350ml bottles at (4500xg=6000rpm) for 20-30 minutes. (save supernatant)

7 ▪ Precipitate the protein from the supernatant by adding slowly the ammonium sulfate (measured and grounded to powder) to 40% (ActA-His) or 60% (ActA-N-His) saturation. (stir in cold room) for 1 hour.

8 ▪ Centrifuge and pellet the precipitated protein at 4500xg for 20-30 minutes.

9 ▪ Re-suspend the precipitated protein in the lysis buffer

Day 3-5

10 ▪ Dialyze the protein in the storage buffer for 3-4 times every six hours to remove the salt before using the Nickle column

Day 6

11 ▪ Purify with Ni-NTA (wash 2 times and elute the protein with the elution buffer). Then, concentrate the elution.

Day 7

12 ▪ Purify the eluted concentrated proteins by Superose-6 (Pharmacia) gel-filtration chromatography.(optional)

When I did the gel-filtration step I lost all my protein.

13 ▪ freeze in N2(l) and stored at 80°C (optional)