**Protein Purification Protocol**

Stock Solutions

20mM Tris-HCl pH 8.0, 15% Glycerol, 150mM NaCl- high salt buffer (to be used for making protease inhibitor and for sonication)

1M NaCl, 50mM Tris-HCl pH=8.0 --- high salt buffer (for column washing)

50mM NaCl, 50mM Tris-HCl pH=8.0 --- low salt buffer

\*Filter sterilize and keep in Fridge\*

In Freezer:

100mM or 1M stocks of IPTG

100mM or 1M stock of Delta-aminoleuvilinic acid (DAC)

300mM Imidazole

Aliquots of BV (wrapped in tin foil)

100 mg/ml Amp

50 mg/ml Kan

Day 1

1. Prep LB media
   1. Add 15.5g of LB powder to create 1L of LB media in a 4L flask
2. Autoclave for 25 minutes on liquid cycle
3. Prep Glassware and Containers
4. Autoclave enough 1L centrifuge bottles
5. Check for all solutions
   1. Kan and Amp
   2. Autoclaved DI water
   3. Low/high salt buffer
   4. DAC and IPTG
   5. Protease inhibitor
   6. Imidazole
   7. BV (in freezer)
6. Plate desired cultures from glycerol stock so that cells are fresh (if needed)

O/N’s

1. Start O/N cultures
2. Add 10 mL LB media with Amp and Kan to 50 mL flask in the presence of a Bunsen burner.
3. Using a loop select one colony from plates and add to the conical tube and stir around real well.
4. Place in incubator overnight at 37°C at 250 rpm – no more than 16hrs

Day 2

1. Grow Up Cells and Induce Gene Expression
   1. To each of the autoclaved 1 L LB media add 1ml of Kan and Amp
      1. Make sure antibiotics are completely thawed before adding (do not use heat) or prepare them fresh
      2. After adding antibiotics, swirl the media for a few seconds
   2. Appropriately label each 1L flask, and add entire sample of O/N cultures to flasks using a pipette
   3. Place flasks in incubator/shaker at 37C and 250 rpm for 2 hrs
   4. Check OD at 600nm
      1. When OD is between 0.4-0.6 proceed with induction of gene expression by adding 5ml of 100mM DAC and 10ml of 100mM IPTG
   5. Change incubator/shaker settings to 20C and 250rpm, and incubate flasks overnight – no more than 16 hrs
2. Prep Talon Columns While Cells Are Growing Up
   1. If preparing new Talon Columns:
      1. To a 10ml disposable gravity column add 1.3 ml Talon resin that is suspended in 20% EtOH
         1. 1.3ml Talon resin per 1L sample
         2. If resin is new, use 1ml

**Note: Everything must move quickly, under sterile conditions, and everything must be kept COLD**

1. Turn on centrifuge to chill to 4°C
2. Obtain enough ice to keep everything cool while working
3. Take Protease inhibitor buffer out of the freezer and thaw
   1. If new protease inhibitor needs to be made: dissolve 1 protease inhibitor pill in 10ml low salt buffer
4. Label all containers and keep them on ice
5. Remove cultures from incubator and pour into 1L bottles and then put them back on ice
   1. Make sure that everything is balanced
6. Put the 1L bottles in the centrifuge
   1. Program: 4000 rpm for 10 minutes
   2. When done there should be a green pellet
7. Decant the supernatant
8. When decanting, pour out the liquid so the pellet is on top
9. Place the bottles upside down on paper towels and blot gently to remove excess media, then place back on ice
10. Add 2mL of low salt buffer in protease inhibitor and resuspend the pellet by mixing with the pipette tip
    1. Use barrier tips
11. Transfer to a 15mL conical tube

Add another 1mL to the area where the pellet was, and mix around to pick up any residual cells, then transfer to the 15mL conical tube

**Protein purification.**

1. Tour the frozen proteins with room temperature water. Leave it for 20 to 30 minutes
2. Sonicate cells
   1. Dip the sonicator pin in the middle of cells
   2. Make sure it doesn’t touch the end and edges or bottle of the conical tube.

1M NaCl, 50mM Tris-HCl pH=8.0 --- high salt buffer (for column washing)

50mM NaCl, 50mM Tris-HCl pH=8.0 --- low salt buffer

\*Filter sterilize and keep in Fridge\*

* + - 1. Open column, let EtOH drip out and resin pack
      2. Wash 3X with dH2O
      3. dH2O should be autoclaved MiliQ water

**Note: water or buffer should never go below the pink resin level. If bubbles appear resuspend the resin**

* + 1. Wash 3X with low salt buffer (50mM NaCl, 50mM Tris-HCl, pH 8.0)
       1. Make sure to leave about a 0.2 cm of buffer above resin surface to prevent it from drying out
       2. Cap the bottom of the column, and then the top
       3. Avoid getting bubbles in the resin, if there are bubbles, resuspend resin
    2. Label and place column in fridge until use
  1. If re-using Talon column, follow steps ii and iii

Day 3

1. This step must be done carefully, pay attention to the heat of the conical tube
2. Rinse sonicator with D.I water, wipe with a kim wipe then rinse with EtOH and wipe with a kim wipe
3. Set “output” to 3.0; duty= varies; timer=4.0 min
4. Do not turn on sonicator until tube is fully inserted
5. Rotate while sonicating in 30 sec intervals, but be careful not to touch the bottom and avoid aeration. Also try not to touch the sides of the conical tube
6. Put on ice and rotate it for 30 sec
7. Repeat sonication 3 more times

**Note: - Should not look like egg whites**

**Sonicating ruptures the cell**

1. Rinse with D.I water before doing a new sample
2. Place back in centrifuge for an hour
   1. Program: 12000 rpm, 4C for 60 min
3. Transfer supernatant to a new 15mL conical tube and add 60µL of 40mM Biliverdin per 1L of cells
   1. Use pipette to mix Biliverdin into solution
   2. All it to sit on ice for 5 min
4. Apply solution to Talon column and resuspend
   1. Place in ice and allow column to settle for about 30 min

**Note: If all of the solution does not fit remember to add it to the column before starting the rinse process**

1. Drain the column without letting anything fall below the resin and without mechanical aid
2. Start washes (mechanical aid can be used at this point)
   1. 5X with low salt buffer
   2. 5X with high salt buffer
   3. 5X with low salt buffer
3. Elute protein in intervals of 300µL of 300mM Imidazole
   1. Label 2 eppendorf tubes
   2. Apply 300µL of Imidazole and collect in first eppendorf tube
   3. Continue to apply 300µL of Imidazole when needed
4. Once drop is green switch to the second eppendorf tube and collect until there is no more green left
5. Removing Imidazole
   1. Add 3 mL of low salt buffer to 50 mL Amicon filtration tube and centrifuge for 20 minutes at 4000 rpm in hanging bucket rotor at 4°C
   2. Apply all protein samples to filter and spin for another 20 minutes
   3. Add another 1mL of low salt buffer to filter and spin again for 20 minutes
      1. Repeat 2X
   4. Remove protein by pipetting (add 100ul of low salt buffer) and place in light-protecting eppendorf tube in refrigerator