

Protein Purification by HPLC (Waters Prep System)

Sample Preparation

- Dissolve 1 L lyophilized protein (typically one Falcon tube) in 4 mL MPW
 - o Keep on ice; store leftovers in -80 freezer
- **Single Sample** (prepare this way if you plan to run only 1-4 injections)
 - o Transfer 400 uL of crude protein to a 15-mL Falcon tube
 - o Add 3200 uL solvent A and 400 uL solvent B
 - o Vortex well to mix
 - o Centrifuge (10 min, 4000 rpm)
 - o Filter sample using syringe filter
 - Remove plunger from 5 mL syringe and attach syringe filter
 - Hold tip of syringe filter over clean 15-mL Falcon tube and add supernatant to barrel of syringe
 - Use plunger to slowly filter liquid
 - Discard filter in trash (can re-use syringe for additional samples)
- **Multiple Samples** (prepare this way if you plan to run 4-8 injections in one day)
 - o Add 32 mL solvent A and 4 mL solvent B to crude
 - o Vortex well to mix, aliquot out into four 15-mL falcon tubes
 - o Centrifuge (10 min, 4000 rpm)
 - o Filter samples using syringe filter
 - Remove plunger from 10 mL syringe and attach syringe filter
 - Hold tip of syringe filter over clean 15-mL Falcon tube and add supernatant to barrel of syringe
 - Use plunger to slowly filter liquid
 - Discard filter in trash (can re-use syringe for additional samples)
 - Repeat until all samples have been filtered

HPLC Start-Up and Column Equilibration

- Check to make sure solvent bottles are at least 1/3rd full
- Turn on computer
- Turn on UV/Vis detector, column heater, and pumps
- Open Breeze software
- Click faucet icon and set flow rate to 10 mL/min solvent B, click apply
- Then click the ramp button and set for 5 min and set pumps to 9 mL/min A, 1 mL/min B, click apply
- After pumps reach 1 mL/min B flow rate, click the "Equilibrate" button on the bottom left. Select "Protein Equilibrate" method and click "Equilibrate/Monitor". Equilibrate column until the baseline is relatively flat (~10 min).