Saptasense Protocol

Large Scale Protein Purification and Buffer Exchange

Background: After inducing protein expression (causing large amounts of the protein to be produced), it is time to purify the protein. We have added a His-tag onto our protein which makes this process considerably easier. Typically His-tag purifications involve loading onto a column, washing, and then eluting with imidazole. However, we have received a His-tag purification system from Promega that will allow us to skip the use of column purification. Instead, their MagneHis technology utilizes magnetic Ni beads which can be collected at the bottom of the eppendorf tubes via a magnetic stand. After collection of the beads at the bottom, the supernatant can be carefully removed and the beads can be resuspended in various buffers. This increases the ease and efficiency of purification.

Materials:

Reagents
MagneHis Kit Components
IPTG
5M NaCl
Microcon Ultra Filter
Enzyme buffer
dH2O

Instruments and Equipment
Teaching lab or Meyer Lab centrifuge
Magnetic stand (we have it)

Procedure:

Cell Lysis

- ☐ Obtain the pelleted sample for purification from the -80°C freezer
- ☐ For every 1 OD600 of the sample, dilute 10uL of FastBreak Cell Lysis Reagent 10X to 100uL by adding 90uL ddH2O
- ☐ Resuspend the cell pellet in the lysis reagent solution you just prepared
- ☐ Add 75 uL lyophilized DNaseI (found in the -20 freezer) to the lysed bacterial culture.
- ☐ Incubate with shaking for 10-20 minutes at room temperature on a rotary mixer or shaking platform.

Purification

- ☐ Create salted wash buffer by adding 50 uL 5M NaCl to 450 uL wash buffer
- ☐ Add 758uL 5M NaCl NaCl to protein lysate. This will improve binding to MagneHis Ni-Particles. Transfer to a 15mL tube
- ☐ Vortex the MagneHis Ni-Particles to a uniform suspension
- ☐ Add 300uL of MagneHis Ni-Particles to the resuspended cell pellet
- ☐ Invert the tube to mix, approximately 10 times. Incubate for 2 minutes at room temperature. Make sure that the particles are well mixed.

- [ ] Place the tube in the appropriate magnetic stand for approximately 30 seconds. Using a pipette, carefully remove the supernatant!
- [ ] Remove the tube from the magnetic stand. Add 166uL of salted wash buffer to the MagneHis Ni-Particles and pipet to mix. Make sure that the particles are resuspended well.
- [ ] Place the tube in the magnetic stand for another 30 seconds. Remove the supernatant carefully. When opening the cap of the tube, it tends to displace the beads. To make things easier I recommend leaving the caps open during the whole washing/eluting process.
- [ ] Repeat the wash step 2 more times for a total of 3 times.
- [ ] Remove the tube from the magnetic stand and add 200uL of MagneHis Elution buffer and pipet to mix.
- [ ] Incubate for 1-2 minutes at room temperature. Place it in the magnetic stand. Remove the supernatant which contains the purified protein!

Buffer Exchange

- [ ] Create 50 mL enzyme buffer
    - [ ] Predetermine the appropriate buffer to store your enzyme in for activity assays and create 50 mL of that buffer
    - [ ] Ex. ChOx buffer: 10 mM EDTA in phosphate buffer pH 7.4
- [ ] Put Microcon column in a microcentrifuge tube
- [ ] Pre-rinse Microcon column with prepared buffer (200uL approx)
- [ ] Fill the column with buffer and centrifuge at 14,000 x g for about 20-40 minutes
    - [ ] Stop centrifuging when the liquid has mostly run through the filter
- [ ] Add purified sample to reservoir of centrifugal device
- [ ] Add buffer to get the total volume to .5 mL
- [ ] Close cap. Place tube in centrifuge with the cap strap facing inwards
- [ ] Centrifuge at 14,000 x g for about 20-40 minutes
    - [ ] Check every 5 minutes that the filter is not dry, as a dry filter will result in lost protein yield
- [ ] Remove filtrate (save until you're sure you have your protein)
- [ ] Add prepared buffer to nearly fill the filter (200uL approx)
- [ ] Centrifuge at 14,000 x g for about 20-40 minutes
    - [ ] Check every 5 minutes that the filter is not dry, as a dry filter will result in lost protein yield
- [ ] Remove filtrate (save until you're sure you have your protein)
- [ ] Repeat the buffer wash and centrifugation
- [ ] Place a clean microcentrifuge tube overtop the filter and invert (so the filter is now upside-down in the tube)
- [ ] Centrifuge at 1,000 x g for 5 minutes
- [ ] Remove the filter. Your protein will be in the filtrate.

- [ ] Add enough buffer to the filtrate to have a total volume of 200 uL
- [ ] Aliquot in 20 uL volumes
- [ ] Retain one aliquot for NanoDrop and SDS PAGE analysis. Flash freeze the rest in liquid nitrogen and store at -80C
- [ ] Determine the enzyme concentration using the NanoDrop
- [ ] Analyze sample using SDS PAGE
- [ ] Example scheme from website: