Stock Solutions:
LB
100mM IPTG (2.38g IPTG in 100mL ddH$_2$O; filter sterilize; store -20° C)
Ampicillin or Carbenicillin (use at 50ug/mL)
Solutions Provided From His-Bind Buffer Kit (Novagen Cat.#69755-1):
• 8x Binding Buffer
• 8x Wash Buffer
• 4x Elute Buffer
• 4x Strip Buffer
• 8x Charge Buffer
Other Novagen Products Used:
• Pre packed His- Bind Column (#70971) stored at 4° C
• Bug Buster (#70584) stored Room Temp.
• Benzonase (#70746) stored at -20° C

Inducing Recombinant Protein Expression and Preparing Cell Lysate
A. Induction
1. Streak Amp-LB plate with freezer stock E63-1xHis BL21 cells and incubate plate overnight
2. Pick single colony from freshly streaked plate and inoculate 5ml of LB-Amp media. Grow overnight
3. Inoculate 100ml of LB-Amp media with 1ml of overnight culture.
4. Incubate in shaker at 37° C until OD$_{600}$ = .6 (usually about 3 hours)
5. Remove 500ul of culture as uninduced control. Add IPTG for final concentration of 0.4mM. Incubate for another 2-3 hours. Remove 500ul aliquots at 30min intervals to monitor protein expression over time; process these samples in the section called “SDS-PAGE of Control Samples”.

B. Cell Extract Preparation
1. Place flask on ice for 5 minutes then make 8-10 10ml aliquots in centrifuge tubes.
2. Centrifuge in Hoshizaki centrifuge at 10 000g for 10min. (6,500 RPM JA-20 rotor or equivalent)
3. Decant supernatant and drain off as much liquid as possible by gently tapping upside down on paper towel.

SELECT ONE TUBE FOR PURIFICATION AND STOR THE OTHERS AT -80° C
4. Resuspend pellet in 2.5ml of room temp. BugBuster/Benzonase (1ul Benzonase per 1ml BugBuster). Gently vortex or pipette to get pellet into solution.
   *note: there are no adverse effects of using larger volumes of BugBuster
5. Incubate the cell suspension on a shaking platform for 10-20 min at Room Temp. The solution should not be viscous at the end of the incubation.
6. Remove insoluble cell debris by centrifugation at 16,000g (~16000rpm in Hoshizaki centrifuge) for 20 min.
7. Transfer the supernatant to a fresh 50 ml tube. Keep tube on ice until ready to be loaded.

**Affinity Chromatography**

A. Column Preparation
1. Remove His-Bind Column cap, pour off or pipette all of the storage buffer in the lower chamber and remove plug.
2. Equilibrate the column with 10 ml of 1x Binding Buffer. Allow the entire buffer volume to flow through the column.

B. Column Chromatography
1. After Binding Buffer has drained, load the column with the prepared cell extract. (Should be ~2.5 ml of solution).
2. Wash the column with 10 ml of 1x Binding Buffer
3. Wash the column with 10 ml of 1x Wash Buffer
4. Elute the protein from the column with 5 ml of 1x Elute buffer. Capture 1 ml fractions in 1.5 ml Eppendorf tubes.

**Processing of Purified Samples**
1. Clamp one end of pre-treated tubing with molecular weight cutoff range of 12,000-14,000 (Fisher Scientific Cat. #08-667A, 1 cm tubing). Pipet eluted protein into dialysis tubing and clamp the other end shut.
2. Place tubing in a large beaker (3 L) filled with cold Tris storage buffer (10 mM Tris-HCl, pH 7.5) and stir on stir plate. Make sure stir bar is not hitting the tubing. Dialyse overnight and change storage buffer at least once.
3. Determine the protein concentration by method of choice. For long term storage keep at -80°C; storage at -20°C seems to be alright.

**SDS-PAGE of Control Samples**
1. Pellet cells from each 500 ul aliquot by centrifuging for 5 min. at 6000 RPM. Remove supernatant and resuspend pellet in 50 ul 1x Laemmli sample buffer. Store at -20°C until samples are ready to use.
2. Heat samples at 70°C for 5 min. and run 5-10 ul on an SDS-polyacrylamide gel (15% for E63-1). Stain with Coomassie Blue 15 – 30 min. Destain with destaining solution to visualize proteins. (destaining may take overnight).