K. Mace, September 2007

His-tagged protein purification under denaturing conditions

Prep: Homogenizer, with 2 100 mL grad cylinders for rinsing
Ice bucket with powdered dry ice
Falcon 2059 tubes (14 mL)
Scalpel and glass plate
Ni-NTA resin (QIAGEN) – prep first by washing aliquot 5X with ULB
Stock Buffer – basis of all (make 900 mL: DO NOT AUTOCLAVE)
100 mM NaH_2PO_4 → 13.8 g NaH_2PO_4-H_20 (MW 137.99)
10 mM Tris-Cl → 1.2 g Tris base (MW 121.1)
8 M Urea → 480.5 g Urea (MW 60.6)
NOTE: Stock Buffer is 900 mL instead of 1 liter so to allow for volume increase with pH adjustment, so for each buffer below, start with 18 mL and bring volume up to 20 mL.
Urea Lysis Buffer (make 20 mL/sample from Stock Buffer)
→ Adjust pH to 8.0 using NaOH immediately prior to use
Wash Buffer (make 20 mL/sample from Stock Buffer)
→ Adjust pH to 6.8 using HCl immediately prior to use
Elution Buffer 5.9 (make 20 mL/sample from Stock Buffer)
→ Adjust pH to 5.9 using HCl immediately prior to use
Elution Buffer 4.5 (make 20 mL/sample from Stock Buffer)
→ Adjust pH to 4.5 using HCl immediately prior to use

1. Keep samples on dry ice until ready, weigh sample in tube
2. Roughly mince tissue with clean scalpel on clean glass plate
3. Place pieces into Falcon 2059 tube and add 1 mL Urea Lysis Buffer/200 mg tissue.
4. Homogenize sample ~15 seconds, rest, repeat (check for completion).
5. Pellet cellular debris 10 minutes at 5Krpm.
6. Optional: store samples until ready for use. Transfer sample to Eppendorfs - aliquot samples into 250 uL each, snap freeze on dry ice and store at -80°.
7. Thaw samples at 37 degrees for 10 minutes. Vortex to mix. Optional: Can load total protein on WB or continue with purification.
8. Wash appropriate amount of Ni-NTA (~200 uL) 2X with 5 mL Urea lysis buffer. Spin 1K for 2 min. each time. Transfer protein samples to washed Ni-NTA in 5 mL total Urea lysis buffer in Falcon tube.
9. Rock at RT 1-4 hours (check for coagulation while incubating!).

10. Spin 10 min at 1K rpm. (Remove remaining supe – optional: save for analysis)
11. Wash Ni-NTA with 5 mL Wash Buffer 2X 1 hr at RT, rocking, spin 10 min at 1K rpm each time. Transfer Ni-NTA to 1.5 mL tube. Can leave protein on Ni-NTA in 50% slurry for WB analysis, or can elute as follows…
12. Elution 1: Elute his-tagged protein from Ni-NTA with 2X 200 uL Elution Buffer 5.9, rocking 1 hr at RT. Spin 10 min at 1K rpm. (Most 6X-his-tagged proteins come off here.)
13. Elution 2: Elute 2X with 200 uL Elution Buffer 4.5, rock 1 hr at RT. Spin 10 min at 1K rpm. (Some his-tagged proteins come off here.)
14. Add 200 uL of EB 5.9 to Ni-NTA to make 50% slurry again for analysis of remainder.
15. Aliquot samples and snap freeze on dry ice. Store at -80°.