**Purifying His-tagged proteins on FPLC**

**Updated November 2011**

**Express protein**

1. Streak fresh plate from glycerol stock. Modified pET28b vector containing His-tag and TEV site is Kanamycin resistant.
2. Pick colony and inoculate 3 mL 2XYT (+3 µl Kan). Grow for 6-8 hrs at 37˚C.
3. Inoculate 25 mL 2XYT (+25 µl Kan) with 50 µl of starter culture. Grow overnight at 37˚C.
4. Autoclave 500 mL TB in baffled flask.
5. In morning, inoculate 500 mL (+500 µl Kan) with 10 mL from overnight. Grow at 37˚C and monitor O.D.600
6. At O.D.600=0.8 (around 2 hrs), induce with 500 µl IPTG.
7. Grow for another 4-6 hrs.
8. Harvest cells by centrifugation (5000 rpm for 15 min).
9. Pour off supernatant and store pellet at -20˚C.

**Prepare cell lysate**

1. Ni column should be run in phosphate buffer, high salt, and low imidazole (to prevent non-specific binding). Sample will be eluted in same buffer but with high imidazole. Make binding and elution buffers:
	1. Binding buffer: 20 mM sodium phosphate, pH 8

500 mM NaCl

20 mM imidazole

* 1. Elution buffer: 20 mM sodium phosphate, pH 8

500 mM NaCl

500 mM imidazole

* 1. Note: if making fresh imidazole stock solution, remember to adjust pH to 8.
	2. Filter and degas buffers. Hook up to FPLC and prime lines (after priming system and column with water).
1. Resuspend pellet in 25 mL of binding buffer. Keep on ice. Transfer to 50 mL conical.
2. Add ½ tablet protease inhibitor (Complete, EDTA-free, Protease inhibitor cocktail tablets from Roche).
3. French press 6-8 times.
4. Spin down in JA-10 rotor with adaptors, at 5500 rpm for 30 min.
5. Carefully pour off and keep supernatant. Add 50 µl of DNase (1 mg/ml stock).
6. Filter through 0.45 µm syringe filter.
7. Take sample for SDS-PAGE.

**Run Ni column (1 ml HisTrap columns from GE)**

1. Equilibrate column in binding buffer. Run at 1 ml/min.
2. Load lysate in sample loop and inject onto column. Wash with at least 4 column volumes of binding buffer.
3. Elute with elution buffer (or a gradient).
4. Re-equilibrate column and perform second injection and elution if necessary.
5. Wash column with water and 20% ethanol for storage.
6. Collect fractions and run SDS-PAGE gel to visualize protein fractions.

**Cleave His-tag with TEV**

1. Pool fractions containing target protein and take absorbance scan to determine concentration.
2. Add appropriate amount of TEV protease (stored in -80˚C).
	1. For easily cleaved proteins (like SurA), use a 1:20 molar ratio of TEV to protein and add the TEV directly to the sample and immediately start dialyzing.
	2. For more difficult proteins (like Skp), use a 1:10 molar ratio, and set up a cleavage reaction mixture with 1 mM EDTA and 5 mM TCEP. Incubate in conical tube for 3 hrs before beginning dialysis.
3. Dialyze protein/TEV mixture overnight at room temperature into binding buffer (20 mM NaP, 500 mM NaCl, 20 mM imidazole).
4. In morning, equilibrate column again in binding buffer and inject sample. With no His-tag, the protein should now come off in the flow-through and wash. Run elution buffer as well to see TEV elute (which is itself His-tagged) and uncleaved protein.
5. Collect fractions and run SDS-PAGE gel to visualize protein fractions.
6. Pool fractions containing cleaved protein and dialyze into desired buffer overnight (for example, 20 mM Tris pH 8). Skp should be dialyzed into Tris and 150 mM NaCl to prevent precipitation.