IMPACT Purification of EF-Tu (Byron Hetrick)

This protocol is for the purification of EF-Tu on one 15mL chitin column. It uses 2L of culture per 15mL column. Typically, yield is about ~14mg (~7mg/L culture).

Each column is good for 4 uses then should be replaced.

Cell culture and protein expression:

- Inoculate 5mL LB/Amp from frozen cell stock.
  - Incubate 37°C shaking overnight (>12hr)
- Inoculate 2X 500mL LB/Amp with 1mL of saturated starter culture.
  - Incubate on 37°C shaker until OD_{600} = 0.5-0.8 (3h)
  - Induce by adding IPTG to final concentration of 0.5mM (60mg for each 500mL culture)
  - Incubate 15°C shaking overnight
- Pellet cells by centrifuging 5,000xg 10min. 4°C
- Store pellet at -20°C or continue to affinity purification.

Affinity purification:

- Prepare the chitin column.
  - Use 15mL chitin beads for 1L of cell culture.
  - Equilibrate with 10 column volumes (CV) of column buffer before using.
- Prepare crude cell extract.
  - Resuspend cell pellet in 40mL cold cell lysis buffer
  - Lyse cells by sonication (8sec on 8sec off for 1min. at 60%. Repeat 3X with 1min rest between each)
  - Save 40µL of total cell extract for gel analysis
  - Centrifuge 20,000xg 30min 4°C (will need to split into 2 oak ridge tubes)
  - Save 40µL of cleared cell extract for gel analysis.
- Load cleared cell extract onto column
  - Flow rate ~0.5-1mL/min.
  - Save 40µL of flow-through for gel analysis.
- Wash the column with 10CV of column buffer (flow rate ~2mL/min)
- Quickly flush column with 3CV of cleavage buffer
- Incubate column for 16hours at room temp.
- Elute column with column buffer
  - Collect 20~30mlmL
- Run SDS-PAGE (5% stacking, 10% separating) of fractions, total cell lysate, cleared lysate, and flow through (load at least 10µL from each fraction)
  - You may also remove some beads from the column and boil then run on gel to determine cleavage efficiency.
- Concentrate fractions containing EF-Tu in concentrator (spin speed and time depends upon concentrator used. EF-Tu is ~45kDa so use a MW cutoff no larger than 30kDa)
  - With most concentrators used, 30min at 4°C and 2,800RPM in the clinical centrifuge works well.
- Wash EF-Tu to a dilution factor of ~200 in concentrator.
  - Example: Concentrate initial fractions down to 1mL. Add 14mL of storage buffer and concentrate down to 1mL. Add 14mL more of storage buffer and concentrate again down to 200-300uL. This will result in a dilution factor of 225 for the column buffer (15^2).
- Final volume of EF-Tu should be 1.5ml. This will typically yield a protein concentration of 200~250µM
- Determine protein concentration by Bradford assay.

- Aliquot and flash freeze. Store at -80°C

Column Regeneration:

- Wash column with 3CV of 0.3M NaOH
- Stop flow, let soak 30min.
- Wash with 7CV of 0.3M NaOH
- Wash with 20CV of water
- Wash with 5CV of column buffer (-GDP) with 0.02% Sodium Azide
- Store column at 4°C. Each column should be used no more than 4 times!

Buffers:

Cell Lysis Buffer

20mM Tris-Cl pH 8.0	(1M)	1mL
500mM NaCL	(5M)	5mL
1mM EDTA	(0.5M)	100µL
0.1% Triton X-100	(100%)	50µL
Final Volume		50mL

Column Buffer

20mM Tris-Cl pH 8.0	(1M)	10mL	2mL
500mM NaCl	(5M)	50mL	10mL
1mM EDTA	(0.5M)	1mL	200µL
30uM GDP	(100mM)	150µL	30uL
Final Volume		500mL	100mL

Clevage Buffer: Add 2.5mL 1M DTT to 47.5mL column buffer

Storage Buffer

50mM Tris-Cl pH 7.5	(1M)	2.5mL
60mM NH_4Cl	(5M)	600µL
1mM DTT	(1M)	50µL
20uM GDP	(100mM)	10µL
Final Volume		50mL

Example of purification gel: (S-supernant, F-flow through, B1-before cleavage, B2-after cleavage, P-purified protein, M-Marker)