**SecA Purification**

(adapted from Donald Oliver's lab procedure, Wesleyan Univ., CT)

**Growth and Induction**

• Inoculate BL21.19 (pCS1) cells in 10 mL LB + Amp and grow overnight at 37°C.

• Inoculate 1 L LB + Amp and grow up to OD600 ≈ 0.6.

• Induce with 0.5 mM IPTG for 1.5 hr.

• Pellet cells. Wash with 50 mL ice-cold TKMD. The cell pellet can be used directly or stored at -80°C. The weight of the cell paste must be known so *tare the centrifuge bottle!*

**Purification** (everything done on ice or at 4°C)

• Resuspend cells in 2 mL TKMD per gram of cell paste. Add 0.5 mM PMSF.

• Pass through French Press twice at 15 kpsi.

• Spin lysed cells 17,500*g* for 20 min in JA-25.50 rotor. and carefully remove s/n;

• Carefully remove supernatant and respin at 135,000*g* (50K in TLA 100.3 rotor) for 3 hr. Remove supernatant and add 0.5 mM PMSF. This supernatant is called S135 (135K supernatant).

• Equilibrate Reactive Blue 4-Agarose (Sigma R-2507) with TMKD (2 mL per 5 mL S135). After final wash, pellet Reactive Blue 4-Agarose and add S135. Rock overnight at 4°C.

• Pellet agarose. Wash with:

a) 2X 20 mL TKPD-low

b) 1X 5 mL TLPD-high

(15 min rocking and 5 min spin for each wash step)

• After final wash, pellet agarose and remove the last drops of buffer. Elute Sec A 2X with 1 mL TKEPB-high, (this volume can be adjusted).

• Dialyse against 2 changes of 500-1000 mL of TKEPB-low.

• Add glycerol to 10-15%. Aliquot, and store at -80°C.

**TKMD: 100 mL**

10 mM Tris-HCl, pH 7.5 1 mL 1 M Tris, pH 7.5

50 mM KCl 2.5 mL 2 M KCl

10 mM MgOAc 1 mL 1 M MgOAc

 1 mM DTT 0.1 mL 1 M DTT

 95.4 mL H2O

**TKPD-low: 100 mL**

 25 mM Tris-HCl, pH 7.5 2.5 mL 1 M Tris, pH 7.5

 50 mM KCl 2.5 mL 2 M KCl

 5 mM DTT 0.5 mL 1 M DTT

0.5 mM PMSF 0.5 mL 0.1 M PMSF

 94 mL H2O

**TKPD-high: 50 mL**

 25 mM Tris-HCl, pH7.5 1.25 mL 1 M Tris, pH 7.5

0.3 M KCl 7.5 mL 2 M KCl

 5 mM DTT 0.25 mL 1 M DTT

0.5 mM PMSF 0.25 mL 0.1 M PMSF

 40.75 mL H2O

**TKEPB-high: 10 mL**

 25 mM Tris-HCl, pH7.5 250 µL 1 M Tris, pH 7.5

1.3 M KCl 6.5 mL 2 M KCl

0.5 mM EDTA 10 µL 0.5 M EDTA

0.5 mM PMSF 50 µL 0.1 M PMSF

 5 mM -ME 3.5 µL -ME

 3.14 mL H2O

**TKEPB-low: 2000 mL**

 25 mM Tris-HCl, pH7.5 50 mL 1 M Tris, pH7.5

 25 mM KCl 25 mL 2 M KCl

0.5 mM EDTA 2 mL 0.5 M EDTA

0.5 mM PMSF 10 mL 0.1 M PMSF

 5 mM -ME 700 µL -ME

 1903 mL H2O