

## **P.69 Pertactin Prep and Initial Purification**

Clark Lab

Kay Finn, Mirco Junker

last updated: January 15, 2003

### Growth:

Streak cells from master stock onto LB-amp plate (freshly made, 100 ug/ml ampicillin).  
Grow at 37°C. Next day, use 1 colony for overnight culture in 100 ml LB-amp, 37°C, shaking.  
Inoculate 4\*1000 ml LB-amp (in 2L baffled flask) with 20 ml of overnight culture each. Grow at 37°C in shaker for 2.5 hrs.

Take OD at A600. Should be 0.6.

Start expression with addition of 2 ml of IPTG (30mg/ml, frozen stock) and continue shaking, 37°C, for 4 hours. Centrifuge at 4°C for 15 minutes at 5000 rpm in GSA bottles.

Freeze over night at -70°C.

### Purification:

Thaw cells in room temperature water bath. Add 25 ml sonication buffer:

..... 50 mM TRIS pH 8.8

.....100 mM NaCl

.....1 mM EDTA

.....10 mM Benzamidine

Sonicate on ice for 6 minutes at 100% power, 30 secs on, 30 sec off with flat probe. (sonicator in Castellino Lab).

Centrifuge at 10,000 rpm for 20 minutes at 4°C.

Remove supernatant.

Wash Pellet with the same volume of sonicating buffer + 1% Triton X-100, spin, remove supernatant.

Solubilize pellet in sonicating buffer + 6 M Guanidine HCl as small a volume as possible. Use a 10 ml pipet to mix pellet with liquid.

Carefully reduce the concentration of GuCl by successive dilutions with buffer without GuCl, in the following manner:

add 1.0x volume = total 2x dil = 3.0 M [GuCl]

add 1.0x volume = total 3x dil = 2.0 M [GuCl]

add 1.0x volume = total 4x dil = 1.5 M [GuCl]

add 1.0x volume = total 5x dil = 1.0 M [GuCl]

Dialyze against 50 mM Tris pH8.8, 100 mM NaCl, 1 mM EDTA using Pierce Slide-A-Lyzer cassettes and loading up to 10 ml in each. Dialyze against 10 L of chilled buffer made from lab stocks, stir overnight in cold room.

Centrifuge contents of dialysis in SS34 tubes, 10,000 rpm for 20 at 4°C

Remove supernatant and dialyze against 50 mM Tris pH8.8 (10 L, cold room). Dialyze again against 50 mM Tris pH 8.8 (10 L, cold room). Sterilize and degas this solution by filtering through a 0.22 um membrane before loading on the column.