

## **PBC Day One Interpretation Questions and Answers**

1. In this experiment, we combine three different methods to break open the *E. coli* cells. What are they, and what is the purpose of each step?

- 1) Cells are subjected to at least 1 cycle of freeze/thaw, which breaks the outer membrane;
- 2) Cells are treated with lysozyme, which hydrolyzes glycosidic linkages in the bacterial cell wall, thereby breaking it down;
- 3) Cells are subjected to a hypotonic environment using the detergent Triton X-100, allowing osmolysis. This ruptures the inner membrane, releasing the cell contents.

2. a) Does total activity increase or decrease during purification? Explain your answer.

**Total activity is the total number of enzyme activity units (U) recovered at each step (and present in the sample from that step). It will usually decrease during purification, because protein is lost in various steps, and protein is also denatured during manipulations.**

2b) Does specific activity increase or decrease during purification? Explain your answer.

**Specific activity is the ratio of activity units to amount of protein (U/mg), which should increase during the purification. During the purification process, lots of undesired proteins are purified away, but the desired protein giving the activity remains, thereby becoming enriched at each step. Thus the ratio of total activity to total amount of protein increases.**

3. Interpret your data, focusing especially on the beta-gal activity assay results for the CL-S and CL-P samples.

- a) What was the total activity of each sample?
- b) Where was the majority of the activity after centrifugation?
- c) You have not yet measured the activity for your ammonium sulfate samples, so instead predict where the majority of the activity will be in the ammonium sulfate samples (supernatant or pellet) and explain why.

### **Sample calculation:**

- $A_{420}$  of reaction mixture =  $A_{420}$  at stop \* 2  
= .159  $A_{420}$  (measured) \* 2  
= .318  $A_{420}$
- Reaction rate in  $A_{420}$ /min:  
= .318  $A_{420}$ /15 min = .0212  $A_{420}$ /min
- Convert to [ONP]/mL/min  
= .0212  $A_{420}$ /min \* (1nmol ONP/ml)/.0045  $A_{420}$   
= 4.711 nmol ONP/mL/min
- Total volume rxn mixture = 1.104 mL  
therefore:  
4.711 nmol ONP/mL/min \* 1.104 mL = 5.20 nmol ONP/min = 5.20 U
- $\square$  5.20 U / 4 uL = 1.3 U/uL
- $\square$  Total activity in 11.5 mL sample:  
= 1.3 U/uL \* 11.5 mL \*  $10^3$  uL/mL  
= 14,950 U

## **PBC Day 1 Interpretation Questions and Answers (continued)**

- **The total activity of each sample will vary student to student.**
- **The majority of activity after centrifugation will be in the crude lysate supernatant. The majority of activity after the ammonium sulfate precipitation will be in the pellet; we are “salting out” (precipitating) the protein using ammonium sulfate.**