

HOMWORK ASSIGNMENT #1

DUE ON WEDNESDAY (1/23/08)

You have isolated 2 bacteria clones, designated GEX and GM2, and would like to compare the levels of recombinant glutathione S-transferase (GST) expressed by each clone. **Procedure:** the bacteria were grown until they reached an $A_{600} = 0.5$; the bacteria suspensions were disrupted to release their contents (ie, proteins); and either 5 μl or 50 μl of these suspensions were used to determine the amount of GST activity in each of the clones. The amount of GST activity is determined according to the following reaction:

glutathione (GSH) + 1-chloro-2,4 dinitrobenzene (CDNB) \rightarrow CDNB-GSH conjugate.

The CDNB-GSH conjugate exhibits a strong absorption at 340 nm with a molar extinction coefficient (ϵ) = $9600 \text{ M}^{-1} \text{ cm}^{-1}$.

The GST activity is measured by mixing the indicated volume of the bacterial suspension (or a blank) with the substrates (GSH and CDNB) and appropriate buffers in a cuvette with a final volume of 1 ml. The appearance of the CDNB-GSH conjugate was measured with a spectrophotometer (wavelength = 340 nm) at 30 second intervals for a total of 5 minutes.

Results (next page): The absolute A_{340} values measured at 30 second intervals for 5 minutes for each of the 5 samples and a graphical representation of the same data is shown. The 5 samples are: 1) blank (sample without bacteria, but containing all of the substrates and buffers), 2) 5 μl of the GEX extract, 3) 5 μl of the GM2 extract, 4) 50 μl of the GEX extract, and 5) 50 μl of the GM2 extract.

1. **How much GST is expressed in GEX and GM2 using the units $\mu\text{moles CDNB-GSH formed per min per ml of bacteria}$ ($\mu\text{moles min}^{-1} \text{ ml}^{-1}$). Show work and explain why you chose the particular data used in your calculations.**
2. **Bacteria are easily quantified by measuring light scatter at 600 nm. If 10^9 bacteria/ml result in an $A_{600} = 1.0$, calculate the enzyme activity of GEX and GM2 expressed in the units of $\mu\text{moles CDNB-GSH formed per min per } 10^6$ bacteria. (A_{600} values are given in the first paragraph.)**

time (min)	blank	GEX (5 μ l)	GM2 (5 μ l)	GEX (50 μ l)	GM2 (50 μ l)
0.0	0.005	0.018	0.022	0.117	0.103
0.5	0.006	0.049	0.031	0.220	0.143
1.0	0.008	0.082	0.040	0.314	0.184
1.5	0.011	0.111	0.049	0.397	0.225
2.0	0.012	0.142	0.056	0.478	0.266
2.5	0.014	0.173	0.065	0.545	0.305
3.0	0.017	0.204	0.074	0.607	0.345
3.5	0.020	0.232	0.083	0.665	0.384
4.0	0.022	0.264	0.093	0.713	0.423
4.5	0.024	0.292	0.101	0.760	0.456
5.0	0.026	0.322	0.110	0.797	0.494

