A SOLUTION TO THE SPECTROPHOTOMETRY HOMEWORK PROBLEM

1. Preliminary examination of the 5 µl and 50 µl samples from both GEX and GM2 suggests that something in the bacteria preparation is inhibiting or interfering with the assay. (The activity in the 50 µl sample is clearly not 10-fold higher than the 5 µl sample.) A potential problem with the 5 µl sample is that the GM2 sample is not much above the blank. This could introduce a larger error term in the calculations. It is important to use the same volume (i.e., conditions) for both samples. I chose the 50 µl samples, although the 5 µl samples are probably more valid since there appears to be an inhibition of activity associated with increasing numbers of bacteria.

2. Examination of the graph of the 50 µl GEX sample indicates that the activity is not linear. This is the result of the substrate being depleted during the assay and a slowing of the reaction rate. (The initial rate of enzyme activity depends on substrate concentration.) Therefore it is necessary to determine the initial slope (e.g., first 0.5 min) of this sample.

3. Calculate the ΔA from the data table (or from the graph):

   \[ ΔA_{\text{blank}} \text{ per 5 min} = 0.026 - 0.005 = 0.021 \]
   \[ ΔA_{\text{GEX}} \text{ per 0.5 min} = 0.22 - 0.117 = 0.103 \text{ (as per step 2)} \]
   \[ ΔA_{\text{GEX}} \text{ per 5 min} = \frac{0.103 \times 10}{10^6 \text{ µmole/mole}} \]
   \[ ΔA_{\text{GM2}} \text{ per 5 min} = 0.494 - 0.103 = 0.391 \]

4. Using Beer's law (A = εdc) and plugging in the known values generates the following equation:

   \[ \text{activity}^* = \frac{(ΔA_{\text{sample}} - ΔA_{\text{blank}})(0.001 \text{ liter})(10^6 \text{ µmole/mole})}{(9600 \text{ liter/mole-cm})(1 \text{ cm})(5 \text{ min})(0.05 \text{ ml})} \]

   OR

   \[ \text{activity} = (ΔA_{\text{sample}} - 0.021) \times 0.417 \text{ µmole/min-ml} \]

(Note there are many ways to solve this problem and it is not necessary to develop such an equation. If the same assay is done routinely, though, such equations are quite convenient.)
5. Plug in the $\Delta A$ values from step 3 into the equation from step 4:

GEX contains **0.42 units** of activity and
GM2 contains **0.15 units** of activity

where a unit of activity equal the $\mu$moles of glutathione transferred per min per ml of bacteria suspension

6. Using the conversion factor, the bacteria suspension contain $5 \times 10^8$ bacteria/ml. To convert the above activity to units per $10^6$ bacteria, divide by $5 \times 10^8$ and multiply by $10^6$ (i.e., divide by 500). The activity is now:

GEX contains **$8.4 \times 10^{-4}$ units** of activity and
GM2 contains **$3 \times 10^{-4}$ units** of activity

where a unit of activity is $\mu$moles of glutathione transferred per min per million bacteria

*Explanation of values in equation # 3:

$\Delta A_{\text{sample}}$ is the change in absorption of the samples being measured for enzyme activity

$\Delta A_{\text{blank}}$ represents the spontaneous formation of product and needs to be subtracted from all of the samples

0.001 liter is the volume in the cuvette expressed in same units as extinction coefficient

9600 liter/mole-cm is the molar extinction coefficient used to convert absorption values to concentration values

1 cm is the thickness of the cuvette (standard size and usually ignored in all calculations)

5 min is the time interval over which the $\Delta A$ is measured. This value must match all of the samples and blanks. If the time intervals are not the same for all of the samples and blanks, then a $\Delta A$/min must be determined for each sample before proceeding with the remainder of the calculation.

0.05 ml is the volume of sample measured. (Typically the equation is also divided by the protein concentration.)