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Exam 1 Solution

METHODS IN CELL BIOLOGY

1. (30 points) Short answer. Briefly define (word, phrase, 1-3 sentences) or answer the following.

   a. How many rpm are needed to centrifuge a sample at 100,000 x g in a rotor with a radius 7.2 cm.

RCF = $1.119 \times 10^{-5} (rpm)^2 r$
$100{,}000 = 1.119 \times 10^{-5} (rpm)^2 7.2$
$(rpm)^2 = 1.24 \times 10^9$
rpm = 35,200

   b. chromophore

A substance that absorbs radiation—especially in the UV/visible range.

   c. β-radiation

An electron ejected during radioactive decay.

   d. What is the lens that magnifies the object in light microscopy?

Objective lens.

   e. molar extinction coefficient

A conversion factor for converting Absorbance into molar concentrations (ε). Specifically, it is equal to the absorbance of 1 M of pure compound.

   f. What is the type of microscopy that permits samples to be 'optically sectioned' through the use of pin holes (apertures) and a movable stage?

confocal

   g. What is the instrument which can evaluate the fluorescence associated with a large number of individual cells relatively quickly?

Flow cytometer

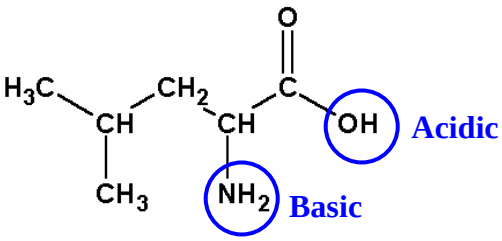
   h. isotope

Different forms of the same chemical element characterized by different atomic masses (or number of neutrons).

   i. What does the "S" in 80S ribosome stand for? [Describe if you do not know the exact answer.]

Svedberg. The unit of measure for the sedimentation coefficient.

j. The structure below has two ionizable groups—one positive and one negative. Circle the two groups and indicate which is positive (i.e., basic) and which is negative (i.e., acidic).



2. (15 points) In honor of Mardi Gras you have selected two groups of mice which have different preferences for rum and coke. The teetotalers refuse to drink it whereas the party animals aggressively seek it out. You are curious about the alcohol dehydrogenase levels between the two groups. A liver is removed from one mouse in each group and homogenized in a total of 5 ml of buffer. 50 μl of each homogenate is then added to 0.95 ml of a solution in a 1 cm cuvette containing NADH and acetaldehyde and the appropriate buffers. The amount of alcohol dehydrogenase is conveniently followed by measuring the disappearance of NADH in the following reaction:

$$\text{acetaldehyde} + \text{NADH} + H^+ \leftrightarrow \text{ethanol} + NAD^+$$

You measure the A_{340} at one minute intervals for a total of 5 minutes. The blank is a cuvette containing the same components as the experimental samples but no liver homogenate. The results are as follows:

	A_{340} values at one minute intervals					
Sample	0	1	2	3	4	5
Blank	0.622	0.618	0.612	0.608	0.604	0.598
Teetotaler	0.619	0.597	0.572	0.551	0.528	0.507
Party Animal	0.623	0.537	0.446	0.372	0.335	0.319

How much total alcohol dehydrogenase activity is found per ml of liver homogenate? (Express answer in μmoles product formed per minute per ml of homogenate). You are concerned about these values since the liver of the party animal was noticeably larger than the liver of the teetotaler and therefore you determine the protein concentration of the homogenates. What is the activity expressed as μmoles product formed per minute per mg liver protein if the homogenates from the teetotalers and party animals had protein concentrations of 2.6 mg/ml and 4.2 mg/ml, respectively?

Plotting the results indicated that the party animal sample was not linear, therefore the Δt of the first minute for all of the samples were used. The following equation was set up to solve the problem:

$$\text{Activity} = \frac{(\Delta A_s - \Delta A_b)(\text{cuvette volume})(10^6\ \mu\text{mole/mole})}{(\varepsilon)(\Delta t)(1\text{ cm})(\text{sample volume})}$$

Plugging in the known values yields this equation:

$$\text{Activity} = \frac{(\Delta A_s - 0.003)(0.001\text{ liter})(10^6\ \mu\text{mole/mole})}{(6.22 \times 10^3\text{ liter/mole}\cdot\text{cm})(1\text{ min})(1\text{ cm})(0.05\text{ ml})}$$

Solving yields:

$$\text{Activity} = (\Delta A_s - 0.003)(3.22\ \mu\text{mole/ml}\cdot\text{min})$$

Note that the ΔA is a negative value since the disappearance of a substrate is being measured. The negative value can be ignored since for every mole of substrate that disappears one mole of product is formed.

The activity per mg protein can be calculated by dividing by the protein concentration:

$$\text{Activity} = (\Delta A_s - 0.003)(3.22\ \mu\text{mole/ml}\cdot\text{min})/(\text{mg protein/ml})$$

Using these equations and substituting in the various values yields these results:

Sample	ΔA_s per 1 min	Act./ml	Act./mg
Teetotaler	0.021	**0.058**	**0.022**
Party Animal	0.086	**0.267**	**0.064**

3. (15 points) You want to make up 100 ml of a 0.1 M Tris buffer with a pH of 8.0. How many grams of Tris base and how many ml of a 1 M HCl (hydrochloric acid = strong acid) solution do you need?

Tris is a base and therefore accepts protons. For every mole of HCl (strong acid) added one mole of Tris is converted to $TrisH^+$ (conjugate acid). The equilibrium can be defined by the following equation:

$$Tris + H^+ \leftrightarrow TrisH^+$$

The total Tris concentration is derived from the free base and is calculated by:

(0.1 moles/liter)(0.1 liters)(121 g/mole) = **1.21 grams Tris**

The pH can be calculated from the H/H equation:

$$pH = pK_a + \log[A^-]/[HA]$$

In this example, HA = $TrisH^+$ and is equal to the amount of HCl added. The A^- will equal the amount of Tris. Remember this is not the total Tris but the Tris in the basic form. So A^- will be equal to the total Tris minus the amount of $TrisH^+$ formed which is equal to the HCl. In other words:

$$[Tris] = 0.1 - [HCl]$$

Substituting these values into the H/H equation:

$$8.0 = 8.3 + \log(0.1 - [HCl]/[HCl])$$

Solving results in [HCl] = 0.067 M

This is the final molar concentration that is needed. To solve for volume use:

$$C_1V_1 = C_2V_2$$

$$(1\ M)V = (0.067\ M)(100\ ml)$$

$$V = (0.067\ M)(100\ ml)/(1\ M) = \mathbf{6.7\ ml}$$

You accidentally add one extra ml (ie, add one to the value obtained above) when making the buffer. How will this affect the pH? [Note: The first part of the question is worth the majority of the points. You can also give a qualitative answer (eg, raises slightly, etc) here for partial credit instead of a quantitative answer.]

Obviously adding an extra ml of HCl to the buffer will lower the pH. The question is how much? This can be calculated by determining the molar concentration of HCl and substituting this value into the equation derived above.

$C_1V_1 = C_2V_2$

C(100 ml) = (1 M)(7.7 ml)

C = (1 M)(7.7 ml)/(100 ml) = 0.077 M HCl

$pH = pK_a + \log(0.1 - [HCl]/[HCl])$

pH = 8.3 + log(0.1 – 0.077)/0.077 = 8.3 + log0.3 = 8.3 – 0.52 = **7.8**

4. (20 points) Answer <u>one</u> of the following two essay questions.

   a. Discuss how the use of a dense medium can facilitate the separation of particles by centrifugation. As part of the answer be sure to identify the 2 major types of density gradient centrifugation and the basis of separation in each case. Briefly describe the conditions under which centrifugation is carried with respect to the particles being separated in each of the two types.

Main points:

- A dense medium can stabilize the sample against vibrations, thermal fluctuations, etc
- A dense medium allows for greater resolution
- 2 types: rate zonal and isopycnic (or equilibrium)
- Rate zonal separates primarily according to mass differences between the solutes or particles
- Isopycnic separates according to differences in density
- Rate zonal is carried out in gradients in which the density is less that the density of the particles being separated. Centrifuge is turned off before particles reach the bottom
- Isopycnic is carried out in densities that encompass the particles being separated. Centrifuge is turned off after system reaches equilibrium—ie, gradient completely formed.

b. DNA can be labeled by incubating cells with radioactive nucleotides which will then be incorporated into the DNA during DNA synthesis. DNA can also be labeled by incubating cells with dyes which fluoresce when bound to DNA. Identify the instruments or techniques that can be used to quantify the DNA with each of these labeling methods. Discuss the advantages and disadvantages of each labeling method. Give examples of when one method might be superior to the other.

Main points:

- ways to measure radioactive DNA: scintillation spectrophotometry, autoradiography (not very good for quantifying)
- ways to measure fluorescence: fluorometry, flow cytometry, fluorescence microscopy (not very good for quantifying)
- radioactive nucleotides require living cells, fixed cells can be used for fluorescent dyes
- radioactivity is better for giving rate of synthesis information
- flow cytometry has advantage of being able to quantify individual cells in a population
- fluorescent dyes will interfere with the structure of DNA whereas labeling with radioactive nucleotides will have little effect