Problem 1. Short answer.

(a) What is the purpose of immunostaining?

The purpose of immunostaining is to detect whether certain proteins are present in a sample of cells.

(b) What are antibodies and antigens? Describe how they are related.

Antibodies and antigens are proteins that bind to each other. Typically, antigens are attached to a target element such as a virus or microorganism. Antibodies are usually generated by an organism's immune system, but they can also be genetically engineered.

(c) What does it mean to fix a cell? Why is it necessary?

To fix a cell means to halt all cellular processes and immobilize proteins. This is necessary to maintain structural stability when lysing the cell membrane. Otherwise, it would be difficult for antibody-antigen interactions to occur.

(d) For Western Blots, the samples are washed with PBS. What reagent do we use to wash the samples in an immunostaining procedure? Why is this necessary?

We use PBS-Tween 20. This is necessary because PBS-Tween 20 prevents non-specific binding. In other words, it ensures that all antibodies are bound to the correct antigen, thus removing background noise.

(e) Why is it important to work in a dimly-lit environment when we are imaging?

It is important because you want to avoid photobleaching. If you work under bright light, the fluorescence won't be detected by the microscope and you can end up with false data.

Problem 2. You are performing an immunostaining lab that requires antigen-X to be diluted by a factor of ξ. If you start out with γ mL of solvent, *approximately* what volume of antigen-X is required?

Denote the volume as V_X, and assume $V_X \ll \gamma$. Also, please express your answer in μL.

Since the problem asks for the approximate volume, let's use the first method described in module:

The problem asks us to express the answer is μL, so first, lets change γ to units of μL:

$$\gamma \text{ mL} = 1000\gamma \text{ μL} \quad (1)$$

Set up a proportion

$$\frac{1}{\xi} = \frac{V_x}{1000\gamma} \quad (2)$$

Solving for V_x, we obtain our final answer:

$$V_x = \frac{1000\gamma}{\xi} \quad (3)$$

Problem 3. Your friend from U$C wanted to run an immunostaining experiment last week, but he realized he ran out of reagents in his lab. As a nice and helpful person, your Principal Investigator offered him some spare antibodies from your lab. After driving over to UCLA and picking up the antibodies, your friend decides to roll down the top of his convertible to enjoy the beautiful Southern California weather on his way back.

The next morning, your friend attempted to perform his immunostaining experiment again. However, the data that he obtained from the microscope was dark and unclear. *Come up with an explanation as to what went wrong. What can he do to fix this?*

He should have taken extra precautions to prevent the sample from being exposed to light.
For instance, he should have:

1) Not roll down the top of his convertible
2) Wrap the sample with foil
3) Store the sample in an opaque container.

At this point, it's too late to fix the problem. The best thing he could do is to restart the experiment, making sure to take the precautions listed.

Problem 4. As an aspiring entrepreneur, you decide to open up a rolled ice-cream shop in Westwood as the COVID-19 pandemic is coming to an end. Of course, the first step to operating a successful business is designing the best recipe for your product.

Unfortunately, your first attempt in creating an ice cream solution was unsuccessful. You realized your solution was too concentrated with Matcha powder. Being the brilliant bioengineer you are, you took out a concentration meter and measured the initial concentration of matcha powder to be $C_{1,\ matcha}$ [mol/L]. You then proceed to find the ideal concentration for matcha in milk. After consulting an online food blog, you learn that you want to bring down the concentration to $C_{2,\ matcha}$ [mol/L]. *In order to reach this concentration, how much of the original solution* V^*_1 [L] *should be added to the milk if you only had* γ [L] *of milk left in your inventory?*

The next week, you came across several Yelp® complaints saying your ice cream was still not tasty. To address this issue, you reached out to one of your customers who also happened to be a food scientist. He suggested that you should add $V_{2,\ honey}$ [L] of $C_{2,\ honey}$ [mol/L] honey syrup to your solution. *What is the total concentration of solute* $C^*_{2,\ total}$ [mol/L] *after this step? Also, what is the total volume of ice cream solution* $V^*_{2,\ total}$ [L] *after this step?* Assume that the initial volume of your $C_{2,\ matcha}$ ice cream solution remained the same despite the fact that a week has passed.

After months of using this new honey-based recipe, the health department decided to conduct a random check-up on your business. They decided that you should bring down the total concentration of matcha and honey to $C_{3,\ total}$. Since your business is booming, you are fortunate enough to have an excess of milk in your inventory. Thus, you decide to combine the entire volume of your current ice cream solution $V^*_{2,\ total}$ with $V^*_{3,\ total}$ ~~[mol/L] of milk from your inventory~~. *Since* $V^*_{3,\ total}$ *is not known, solve for this value in terms of the known parameters.* Assume the volume of milk in your inventory is much greater than $V^*_{2,\ total}$. Also assume that the volume and concentration of the ice cream solution before this step ($V^*_{2,\ total}$ and $C^*_{2,\ total}$) remained the same as what you derived in the previous two questions, despite the fact that months have passed and that business is booming. Note that all variables with an asterisk (*) in this problem are not known.

There is a small mistake in the wording of this. The original worksheet contains the most updated question. However, the solutions are still the same.

Have fun! :))

The first part asks for the amount of original solution to be added (V_1^*). We can use the dilution equation:

$$M_1 V_1 = M_2 V_2 \quad (1)$$

M_1 is given as $C_{1,\ matcha}$ and M_2 is given as $C_{2,\ matcha}$. The total final volume V_2 is given by adding V_1^* to the γ L of milk.

$$V_2 = V_1^* + \gamma \quad (2)$$

Continued...

Combining equations (1), (2), and known values, we obtain:

$$(C_{1,matcha})(V_1^*) = (C_{2,matcha})(V_1^* + \gamma) \qquad (3)$$

Solving for V_1^*:

$$V_1^*(C_{1,matcha} - C_{2,matcha}) = C_{2,matcha}\gamma$$

$$\boxed{V_1^* = \frac{C_{2,matcha}\gamma}{C_{1,matcha} - C_{2,matcha}}} \qquad (4)$$

The next part asks us to find the total concentration and volume ($C_{2,tot}^*$ and $V_{2,tot}^*$) after honey is added. These can be found by logically thinking about the situation.

$$\boxed{C_{2,tot}^* = C_{2,matcha} + C_{2,honey}} \qquad (5)$$

$$V_{2,tot}^* = V_1^* + \gamma + V_{2,honey} \qquad (6)$$

Combining equations (4) and (6):

$$V_{2,tot}^* = \frac{C_{2,matcha}\gamma}{C_{1,matcha} - C_{2,matcha}} + \gamma + V_{2,honey}$$

Continued...

$$V^*_{2,tot} = r\left[\frac{C_{2,matcha}}{C_{1,matcha} - C_{2,matcha}} + 1\right] + V_{2,honey} \qquad (7)$$

The last part of the problem is another dilution question. We want to solve for $V^*_{3,tot}$, which is the final total volume of the solution that we have. Substituting known values into equation (1):

$$\left(C^*_{2,tot}\right)\left(V^*_{2,tot}\right) = \left(C_{3,tot}\right)\left(V^*_{3,tot}\right)$$

$$\left(C_{2,matcha} + C_{2,honey}\right)\left(r\left[\frac{C_{2,matcha}}{C_{1,matcha} - C_{2,matcha}} + 1\right] + V_{2,honey}\right) = \left(C_{3,tot}\right)\left(V^*_{3,tot}\right) \qquad (8)$$

Finally, we solve for $V^*_{3,tot}$:

$$V^*_{3,tot} = \frac{1}{C_{3,tot}}\left(C_{2,matcha} + C_{2,honey}\right)\left(r\left[\frac{C_{2,matcha}}{C_{1,matcha} - C_{2,matcha}} + 1\right] + V_{2,honey}\right)$$