Buffers for Biological Systems Laboratory Instructor's Manual

This instructor's manual is avaliable online only.

1. Purpose and Concepts Covered

This introductory buffers laboratory is for use in undergraduate or highschool courses that cover basic topics in molecular biology or biochemistry. In this manual we include laboratories to illustrate basic chemistry concepts related to buffers and their functions. This is a math-intensive laboratory designed to have students gain comfort with common laboratory calculations.

2. Effect of Temperature and Concentration on pH

Materials Required (for each lab group)

- pH meter and standard buffer solutions
- Tris base (Cat.# H5133)
- 1 M HCl
- glass pipettes
- appropriate eye wear, gloves and other safety protection
- 500 mL glass beaker (per laboratory group)
- nine 200 mL glass flasks (per laboratory group)
- sterile, distilled water
- magnetic stirring plate
- magnetic stirring rods
- 500 mL graduated cylinder (glass)

Always wear appropriate safety clothing and eye protection when working with acids and bases.

Never add water to acid; always add acid to water, slowly!

2.A. Preparing Buffers

1. Prepare 300 mL of a 0.5 M solution of Tris-HCl (pH 9.0).

   Note to Instructor: If students have never before prepared a solution, you may need to walk them through the grams-to-moles conversion.

   Formula weight: 121.14

   121.14 g/L = 1 M solution

   60.57 g/L = 0.5 M solution

   18.17 g/300 mL = 0.5 M solution

   Dissolve 18.17 g of Tris base in 200 mL water. Use the 1M HCl, dropwise, until the pH reaches 9.0. Make sure that all of the Tris base is in solution. Pour into a graduated cylinder. Bring to a final volume of 300 mL with water. Double check to ensure pH is still 9.0.

2. Divide the stock Tris buffer into three 100 ml aliquots. Label as “0.5M Tris buffer (pH 9.0) A”, “0.5M Tris buffer (pH 9.0) B” or “0.5M Tris buffer (pH 9.0) C”.

3. For each aliquot, A, B and C, create a dilution series such that you have the 0.5M stock, 100 mL of 50 mM dilution and 100 mL of 5 mM dilution. Label each flask appropriately.

   Note to the instructor: This is a simple serial dilution exercise, and it provides an opportunity for students to gain confidence manipulating numbers and units. Students will be expected to work between M and mM in order to figure out what kind of serial dilution to perform.

4. Place series A at 25°C for 25 hour. Place series B at 4°C for 24 hours, and place series C at 37°C for 24 hours.

5. After 24 hours, record the pH of the solutions in each of the flasks. If you are using a pH meter that is at room temperature (~25°C), measure the pH of your series B and C flasks one at a time. Do not let them equilibrate to room temperature. Record your results in the table below or using an Excel® spreadsheet or other software.

Concentration	Series A 25°C	Series B 4°C	Series C 37°C
0.5 M			
50 mM			
5 mM			

2.B. Analysis and Discussion

Changes in temperature can dramatically affect pH, particularly in buffers containing reactive amine groups.

Dilution can affect dissociation as well. The pH of Tris decreases approximately 0.1 pH unit per tenfold dilution, and the effect of dilution on pH is more pronounced if you are working at the limits of the buffering range of your system.

Questions for Students

1. Did you notice a change in pH with a change in temperature? Can you explain why temperature would affect pH?
2. Did you notice a change in pH upon dilution of the Tris buffer, even when temperature was maintained?
3. Would you expect to see pH shifts with temperature changes when using a glycine buffer? Why or why not?

3. Exploring Salt Interactions

When salt solutions are mixed, one of two things can happen. There can be no reaction, and all of the ions will remain soluble, or a solid will form (precipitation). When determining what buffer to use for a biochemical system, considering the ions involved is critical. Some buffers can interact with ions in your system, forming precipitates that compromise the reaction you are interested in studying. In this exercise you will examine which salts are soluble and insoluble.

3.A. Determining Rules of Solubility .

Materials Required (for each lab group)

- 1 paper grid (see following page)
- 1 acetate sheet
- masking tape
- the following solutions: 0.1M $AgNO_3$, 0.1M $NaNO_3$, 0.1M $Na_3PO_4 \bullet 12\ H_2O$, 0.1M K_2CO_3, 0.1M $FeCl_3 \bullet 6H_2O$, 0.1M $CuSO_4 \bullet 5\ H_2O$.
- glass pipettes or eye droppers

1. Place your grid on the lab bench. Cover the grid with acetate, and secure it to the bench with tape.
2. Using dedicated droppers for each of the solutions, carefully add one drop (from a height of 1–2 cm) to each box for that solution. For instance, add a drop of $AgNO_3$ to each box in the column underneath the header "$AgNO_3$". Continue until you have a mixture of two solutions in each of the boxes of your grid.

   Note: Do not touch the pipette to the acetate. If you touch the tip of the dropper to another solution, you will contaminate your solution and compromise your results.
3. Observe what is happening in each of the boxes. Some will contain a precipitate (solid) that could be white or another color. Others will remain clear.
4. In the table at the end of this exercise, record your observations for each box in your grid.
5. Carefully remove the tape from the acetate. Rinse off the acetate in the sink. Clean your lab area as instructed.

Math-Intensive Upgrade: Have the students prepare each of the 0.1M solutions used in the laboratory from the stock powders, calculating the grams-to-moles conversions using the MW or FW of each of the powders.

	$AgNO_3$	$NaNO_3$	Na_3PO_4	K_2CO_3	$FeCl_3$
$CuSO_4$					
$FeCl_3$					
K_2CO_3					
Na_3PO_4					
$NaNO_3$					

Promega

3.B. Analysis and Discussion

1. Write a balanced equation representing what you observe in the spaces of the grid where you see a precipitate formed.
2. Which negative ions did not form any precipitate?
3. Which positive ions did not form any precipitate?
4. Which negative ions usually or always form a precipitate?
5. Which negative ions usually or always form a precipitate?
6. If you are studying a reaction in which Ca^{2+} is a required cofactor, how might that be affected by use of a phosphate buffer?

	$AgNO_3$	$NaNO_3$	Na_3PO_4	K_2CO_3	$FeCl_3$
$CuSO_4$	precipitate	no rxn	precipitate	precipitate	precipitate
$FeCl_3$	precipitate	no rxn	precipitate	precipitate	
K_2CO_3	precipitate	no rxn	no rxn		
Na_3PO_4	precipitate	no rxn			
$NaNO_3$	no rxn				

4. Materials Required

Product	Size	Cat.#
Tris Base, Molecular Biology Grade	100g	H5133

From Sigma-Aldrich

Product	Cat.#
$AgNO_3$	2091939-25G
$NaNO_3$	S5506-250G
$Na_3PO_4 \cdot 12H_2O$	04277-1KG
K_2CO_3	P5833-500G
$FeCl_3 \cdot 6H_2O$	157740-5G
$CuSO_4 \cdot 5H_2O$	C8027-500G