
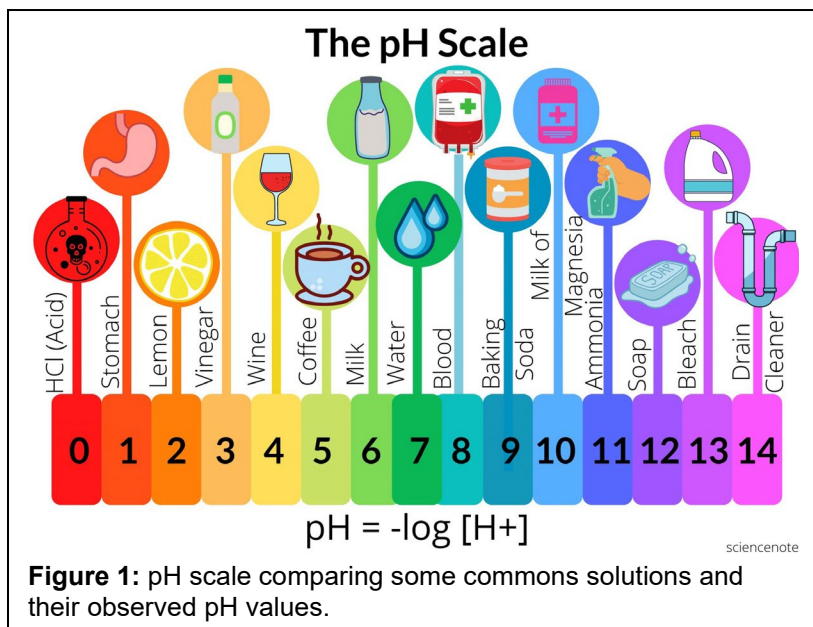
	<b>SKILLS CENTER STANDARD OPERATING PROCEDURE</b>	<b>A BIOFIZZ</b>  <b>PRODUCTON</b>
<b>Making Buffers</b>  <b>Module Hours: 3.0</b>	<b>Effective Date: 04/12/2021</b> <b>PRQs</b> <b>Lab Safety</b> <b>Pipetting</b>	<b>Revision # 2.0</b> <b>M. Stowell</b> <b>Checked: M. Guise, Z. Hazlett-Klein</b>

## BACKGROUND

Most biological experiments require preparation of chemical solutions in their procedure. This is to ensure biomaterials are maintained at physiological conditions for measurements, as well as to alter the charge properties of biomolecules to allow efficient isolation using methods such as ion exchange chromatography or centrifugation. There are two principal methods for the preparation of these chemical solutions; by weight volume (w/v) and by volume volume (v/v). It is important to recognize the difference between these methods and the manner by which such solutions are prepared. Many experiments involving chemicals call for their use in solution form at a defined pH, Figure 1. That is, two or more substances are mixed together in known quantities and the pH is adjusted to a desired value. This may involve weighing a precise amount of dry material or measuring a precise amount of liquid and adjusting the pH accordingly. Preparing solutions accurately improves an experiment's safety and reproducibility.





### 1. PURPOSE

The purpose of this procedure is to understand the basic principles of weighing solid, measuring liquids and adjusting the pH of solutions and to be able to conduct the process in the lab.

### 2. SCOPE

This procedure applies to qualified skills center users.

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### 3. RESPONSIBILITY



- 3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
- 3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
- 3.3. It is the responsibility of the user to become trained on the procedure.

### 4. DEFINITIONS

- 4.1. Solvent - The substance which dissolves another to form a solution. For example, in a sugar and water solution, water is the solvent; sugar is the solute.
- 4.2. Solution - A mixture of two or more pure substances. In a solution, one pure substance is dissolved in another pure substance homogeneously. For example, in a sugar and water solution, the solution has the same concentration throughout, ie. it is homogenous.
- 4.3. Mole - A fundamental unit of mass (like a "dozen" to a baker) used by chemists. This term refers to a large number of elementary particles (atoms, molecules, ions, electrons, etc) of any substance. 1 mole is  $6.02 \times 10^{23}$  molecules of that substance. (Avogadro's number).
- 4.4. pH - The pH scale can tell if a liquid is more acidic or basic. The range of the pH scale is from 0 to 14 from very acidic to very basic. A pH of 7 is neutral. A pH less than 7 is acidic and greater than 7 is basic. pH is a log scale. Each whole pH value below 7 is ten times more acidic than the next higher value. For example, a pH of 4 is ten times more acidic than a pH of 5 and a hundred times ( $10 \times 10$ ) more acidic than a pH of 6. This holds true for pH values above 7, each of which is ten times more basic (also called alkaline) than the next lower whole value. An example would be a pH of 10 is ten times more alkaline than a pH of 9.

### 5. MATERIALS/EQUIPMENT



- 5.1. Deionized water
- 5.2. Solid Buffer Salts
- 5.3. NaOH solution
- 5.4. HCl solution
- 5.5. Graduated cylinders

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- 5.6. Digital scale
- 5.7. pH meter

## 6. RECIPES

- 6.1. 1.5 M Tris-HCl (pH 8.8)  
Tris base 181.7g in 1L H<sub>2</sub>O, adjust pH to 8.8 using HCl.
- 6.2. 0.5 M Tris-HCl (pH 6.8)  
Tris base 60.6g in 1L H<sub>2</sub>O, adjust pH to 6.8 using HCl.
- 6.3. 10% (w/v) SDS  
SDS (sodium dodecyl sulfate) 100g in 1L H<sub>2</sub>O.
- 6.4. 2X SDS Protein Sample Buffer  
1.25M Tris HCl (pH 6.8), 4% (w/v) SDS, 20% (v/v) glycerol, trace bromophenol blue. Note: For reducing conditions use 950 ul 2x SDS sample buffer plus 50µL 2-mercaptoethanol.
- 6.5. 10% (w/v) Ammonium Persulfate (APS)  
10g ammonium sulfate in 100ml H<sub>2</sub>O, aliquot into 1ml vials and stored at -20°C until needed.
- 6.6. Acrylamide/ bisacrylamide stock solution  
For 1L, 30% (w/v) acrylamide 300g, 0.8 % (w/v) bisacrylamide 8g. Add H<sub>2</sub>O to the final volume and filter through the 0.22 µm membrane. Store at 4°C in the dark.
- 6.7. Coomassie Brilliant Blue Staining Solution (1 liter)  
Coomassie Brilliant Blue R250 2.5g, Methanol 400ml, Glacial Acetic Acid 100ml, H<sub>2</sub>O 500 ml
- 6.8. High Methanol Destain Solution  
For 1L, Methanol 400ml, Glacial Acetic Acid 100ml, H<sub>2</sub>O 500ml
- 6.9. 4% (v/v) Glycerol Solution  
For 1L, Glycerol 40ml, H<sub>2</sub>O 960ml

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6.10. Electrophoresis Buffer (TAE)

50X stock solution pH ~8.5, 242g Tris base, 57.1ml glacial acetic acid, 37.2g Na<sub>2</sub>EDTA.2H<sub>2</sub>O, Add distilled water to 1 liter, make 0.5µg / ml Ethidium bromide

6.11. 10X DNA Loading Buffer

20% (w/v) Ficoll 400, 0.1M Na<sub>2</sub>EDTA, pH 8.0, 1.0% SDS, 0.25% bromophenol blue, 0.25% xylene cyanol

6.12. 50 mM CaCl<sub>2</sub> solution, ice cold

6.13. 1 M NaH<sub>2</sub>PO<sub>4</sub> (monobasic)<sup>2</sup>

Dissolve 138 g of NaH<sub>2</sub>PO<sub>4</sub>•H<sub>2</sub>O (monobasic; m.w. = 138) in sufficient H<sub>2</sub>O to make a final volume of 1 L.

6.14. 1 M Na<sub>2</sub>HPO<sub>4</sub> (dibasic)<sup>2</sup>

Dissolve 142 g of Na<sub>2</sub>HPO<sub>4</sub> (dibasic; m.w. = 142) in sufficient H<sub>2</sub>O to make a final volume of 1 L.

## 7. PREPARATION OF SOLUTIONS

7.1. Solution 1. Using percentage by weight (w/v)

7.2. Formula, the formula for weight percent (w/v) is: [Mass of solute (g) / Volume of solution (ml)] x 100.



7.3. Example. A 10% NaCl solution has 10g of sodium chloride dissolved in 100ml of solution.

7.4. Procedure Weigh 10g of sodium chloride. Pour it into a graduated cylinder or volumetric flask containing about 80ml of water. Once the sodium chloride has dissolved completely (swirl the flask gently if necessary), add water to bring the volume up to the final 100ml. Caution: Do not simply measure 100ml of water and add 10g of sodium chloride. This will introduce error because adding the solid will change the final volume of the solution and throw off the final percentage.



7.5. Solution 2: Using percentage by volume (v/v). When the solute is a liquid, it is sometimes convenient to express the solution concentration as a volume percent.

7.6. Formula. The formula for volume percent (v/v) is: [Volume of solute (ml) / Volume of solution (ml)] x 100.

7.7. Example. Make 1000ml of a 5% by volume solution of ethylene glycol in water.

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- 7.8. Procedure. First, express the percent of solute as a decimal: 5% = 0.05. Multiply this decimal by the total volume:  $0.05 \times 1000\text{ml} = 50\text{ml}$  (ethylene glycol needed). Subtract the volume of solute (ethylene glycol) from the total solution volume:  $1000\text{ml}$  (total solution volume) -  $50\text{ml}$  (ethylene glycol volume) =  $950\text{ml}$  (water needed). Dissolve  $50\text{ml}$  ethylene glycol in a little less than  $950\text{ml}$  of water. Now bring the final volume of solution up to  $1000\text{ml}$  with the addition of more water. (This eliminates any error because the final volume of the solution may not equal the calculated sum of the individual components). So,  $50\text{ml}$  ethylene glycol/ $1000\text{ml}$  solution  $\times 100 = 5\%$  (v/v) ethylene glycol solution.
- 7.9. Solution 3: Molar Solutions. Molar solutions are the most useful in chemical reaction calculations because they directly relate the moles of solute to the volume of solution.
- 7.10. Formula. The formula for molarity (M) is: moles of solute / 1 liter of solution or gram-molecular masses of solute / 1 liter of solution.
- 7.11. Example. The molecular weight of a sodium chloride molecule (NaCl) is 58.44, so one gram-molecular mass (=1 mole) is 58.44g. We know this by looking at the periodic table. The atomic mass (or weight) of Na is 22.99, the atomic mass of Cl is 35.45, so  $22.99 + 35.45 = 58.44$ . Or more simply, just look at the container the chemical is sold in or on the Material Safety Data Sheet (MSDS) for molecular weight. If you dissolve 58.44g of NaCl in a final volume of 1 liter, you have made a 1M NaCl solution, a 1 molar solution.
- 7.12. Procedure. To make molar NaCl solutions of other concentrations dilute the mass of salt to 1000ml of solution as follows: 0.1M NaCl solution requires  $0.1 \times 58.44 \text{ g of NaCl} = 5.844\text{g}$ . 0.5M NaCl solution requires  $0.5 \times 58.44 \text{ g of NaCl} = 29.22\text{g}$ . 2M NaCl solution requires  $2.0 \times 58.44 \text{ g of NaCl} = 116.88\text{g}$
- 7.13. Solution 4: Sodium Phosphate buffer. Some buffers are prepared by adding two equal concentration solutions of similar types together to reach the proper buffering range and pH. The preparation of a sodium phosphate buffer is one example of this.
- 7.14. Formula. 1M monobasic ( $\text{NaH}_2\text{PO}_4$ ) and 1M dibasic ( $\text{Na}_2\text{HPO}_4$ ) sodium phosphate solutions must be prepared first (See recipes above). To reach the desired pH of a 1M sodium phosphate buffer, follow the table below.<sup>2</sup>

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7.15. Example. You need a sodium phosphate buffer pH 6.7. Prepare 1M solutions of both mono- and di-basic sodium phosphate (See recipes). Add 565 ml 1 M  $\text{NaH}_2\text{PO}_4$  to 435 ml 1 M  $\text{Na}_2\text{HPO}_4$  in a sterile flask.

7.16. Procedure. Prepare 1M solutions of both mono- and di-basic sodium phosphate as directed in recipes. Determine desired pH of final buffer. Add appropriate amounts of mono- and di-basic sodium phosphate to final volume of 1L in sterile flask.

Volume (mL) of 1 M $\text{NaH}_2\text{PO}_4$	Volume (mL) of 1 M $\text{Na}_2\text{HPO}_4$	Final pH
877	123	6.0
850	150	6.1
815	185	6.2
775	225	6.3
735	265	6.4
685	315	6.5
625	375	6.6
565	435	6.7
510	490	6.8
450	550	6.9
390	610	7.0
330	670	7.1
280	720	7.2

Table 1. Volumes of sodium phosphate monobasic ( $\text{NaH}_2\text{PO}_4$ ) and dibasic ( $\text{Na}_2\text{HPO}_4$ ) that must be combined for the desired pHs shown in right column.<sup>2</sup>



## 8. MEASURING PH

8.1. With test papers - Commercial calibrated test papers which are impregnated with pH indicators. The pH is determined by immersing the strip in the liquid to be tested and comparing its color with a standard color chart provided with the pH paper.

8.2. With digital readout via electronic meter - Electronic, bench top meters are available that read pH to resolutions of 0.001. These easy-to-use meters feature built-in memorized buffer values for quick, "automatic" calibration and automatic temperature compensation which eliminates errors in pH measurement caused by solution temperature variations.

8.3. Electronic pH meters require periodic calibration using standard value calibration solutions (sometimes called buffer solutions). Typical standards are pH 4.01, pH 7.01 and pH 10.01.

8.4. To calibrate the pH meter, begin by removing the protective cover from the electrode and immersing the electrode in a container containing the pH 7.0 buffer solution.

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- 8.4.1. Use the “pH/mv” button to change measurement modes; change to pH mode.
- 8.4.2. Make sure to clear existing buffers (displayed as small numbers beneath pH value) when doing a new calibration. Select the “setup” button until “Clear Buffers” is displayed, and then select the “enter” button to clear the buffers.
- 8.4.3. Now, once the pH has reached a steady number, select “standardize” and then “enter”.
- 8.4.4. Make sure to spray electrode with DI water between measurements. Do not wipe electrode.

## 9. TROUBLESHOOTING



- 9.1. pH is not correct: Make sure that the pH meter is calibrated.
- 9.2. When titrating with HCl or NaOH I keep overshooting the target pH: Try using a lower concentration of HCl or NaOH as you titrate closer to the target pH.
- 9.3. I cannot weigh out very small quantities of buffer components: Make a more concentrated stock solution and then pipette the stock solution to achieve your target concentration.

## 10. REFERENCES

1. Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999
2. 1 M sodium phosphate buffer (pH 6.0–7.2). Cold Spring Harbor Protocols. Retrieved from: <http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8303>

## 11. MODULE MASTERY TASK

This task will test your ability to make several standard laboratory buffers with the correct pH.

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1. Create a table showing the quantities (volume/ weight) of every component of the following buffers

Buffer 1. 50 mL of 0.2M Na<sub>2</sub>HPO<sub>4</sub>, 100mM NaCl, pH 7.9

Buffer 2. 25 mL of 0.1M TRIS, 50 mM NaCl, pH 8.1

Buffer 3. 75 mL of 0.1M HEPES, 80mM NaCl, 1% glycerol, pH 7.4

Buffer 4. 100 ml of 1M Sodium Phosphate buffer, pH 7.0 made from stock NaPO<sub>4</sub> and Na<sub>2</sub>PO<sub>4</sub> solutions.

2. Make each of the buffers listed, label them with your name, date and the buffer components. Provide a copy of your table listing the components and amounts and arrange for a proctor to test your buffers.