C2 Biotechnologies, LLC

Using biotechnology to improve the quality of life

SOP-110

Buffers, Solutions & pH

Procedures used to prepare buffers and solutions.

Section 1 Materials and Reagents

1.5 M Tris-HCl (pH 8.8)

Tris base 181.7 g in 1 L H_2O, adjust pH to 8.8 using HCl.

0.5 M Tris-HCl (pH 6.8)

Tris base 60.6 g in 1L H_2O, adjust pH to 6.8 using HCl.

10 % (w/v) SDS

SDS (sodium dodecal sulfate) 100g in 1L H_2O.

2X SDS Protein Sample Buffer

0.25 M Tris Hcl (pH 6.8)

4 % (w/v) SDS

20 % (v/v) glycerol

trace bromphenol blue

Note: For reducing conditions use 950 ul 2x SDS sample buffer plus 50 2-mercaptoethanol.

10 % (w/v) Ammonium Persulfate (APS)

10 g ammonium sulfate in 100 ml H_2O, aliquot into 1 ml vials and store at –20 C until needed.

TEMED

Acrylamide/bisacrylamide stock solution

For 1 L

30 % (w/v) acrylamide	300 g
0.8 % (w/v) bisacrylamide	8 g

Add H_2O to final volume and filter through 0.22 um membrane. Store at 4 C in the dark.

Coomassie Brilliant Blue Staining Solution

(1 liter)

Coomassie Brilliant Blue R250 2.5 g

Methanol	400 ml
Glacial Acetic Acid	100 ml
H_2O	500 ml

High Methanol Destain Solution

(1 liter)

Methanol	400 ml
Glacial Acetic Acid	100 ml
H_2O	500 ml

4 % (v/v) Glycerol Solution

(1 liter)

Glycerol	40 ml
H_2O	960 ml

Electrophoresis Buffer (TAE)

50X stock solution pH ~8.5

242 g Tris base

57.1 ml glacial acetic acid

37.2 g $Na_2EDTA.2H_20$

Add distilled water to 1 liter, make 0.5 ug / ml Ethidium bromide

10X DNA Loading Buffer

20% (w/v) Ficoll 400

0.1 M Na_2EDTA, pH 8.0

1.0% SDS

0.25% bromphenol blue

0.25% xylene cyanol

50 mM $CaCl_2$ solution, ice cold

HS medium:

Spizizen´s medium supplemented with

- 0.5% glucose
- 50 µg/ml DL-tryptophane
- 50 µg/ml uracil
- 0.02% casein hydrolysate
- 0.1% yeast extract [Difco]
- 8 µg/ml arginine
- 0.4 µg/ml histidine
- 1 mM $MgSO_4$

LS medium:

Spizizen´s medium supplemented with

- 0.5% glucose
- 5 µg/ml DL-tryptophane
- 5 µg/ml uracil
- 0.01% casein hydrolysate
- 0.1 % yeast extract [Difco]
- 1 mM $MgSO_4$
- 2.5 mM $MgCl_2$
- 0.5 mM $CaCl_2$

10x Spizizen`s medium:

- 2 g $(NH_4)_2SO_4$
- 14 g K_2HPO_4
- 6 g KH_2PO_4
- 1 g sodium citrate
- add 100 ml distilled water

• autoclave or filter sterilize with 0.25 um filter

• then add 0.1 ml 1 M $MgSO_4$

Antibiotics

Ampicilian (100 mg / ml)

Chloramphenical (20 mg / ml EtOH)

Neomycin (50 mg / ml)

IPTG (1 M stock solution)

Section 2

Protocol

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Preparing Chemical Solutions

Lab experiments and types of research often require preparation of chemical solutions in their procedure. We look at preparation of these chemical solutions by weight (w/v) and by volume (v/v). The glossary below cites definitions to know when your work calls for making solutions:

Solute - The substance which dissolves in a solution.

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.

Solution - A mixture of two or more pure substances. In a solution one pure substance is dissolved in another pure substance homogenously. For example, in a sugar and water solution, the solution has the same concentration throughout, ie. it is homogenous.

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 6.02×10^{23} molecules of that substance. (Avogadro's number).M

Preparation of solutions

Many experiments involving chemicals call for their use in solution form. That is, two or more substances are mixed together in known quantities. This may involve weighing a precise amount of dry material or measuring a precise amount of liquid. Preparing solutions accurately will improve an experiment's safety and chances for success.

Solution 1: Using percentage by weight (w/v)

Formula

The formula for weight percent (w/v) is: [Mass of solute (g) / Volume of solution (ml)] x 100

Example

A 10% NaCl solution has ten grams of sodium chloride dissolved in 100 ml of solution.

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 100 ml.

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.

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.

Formula

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

Example

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

Procedure

First, express the percent of solute as a decimal: 5% = 0.05

Multiply this decimal by the total volume: 0.05 x 1000ml = 50ml (ethylene glycol needed).

Subtract the volume of solute (ethylene glycol) from the total solution volume:

1000ml (total solution volume) - 50ml (ethylene glycol volume) = 950ml (water needed)

Dissolve **50ml** ethylene glycol in a little less than **950ml** of water. Now bring final volume of solution up to **1000ml** 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, 50ml ethylene glycol / 1000ml solution x100 = 5% (v/v) ethylene glycol solution.

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.

Formula

The formula for molarity (M) is: moles of solute / 1 liter of solution or gram-molecular masses of solute / 1 liter of solution.

Examples

The molecular weight of a sodium chloride molecule (NaCl) is 58.44, so one gram-molecular mass (=1 mole) is 58.44 g. 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 on 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.

Procedure

To make molar NaCl solutions of other concentrations dilute the mass of salt to 1000ml of solution as follows:

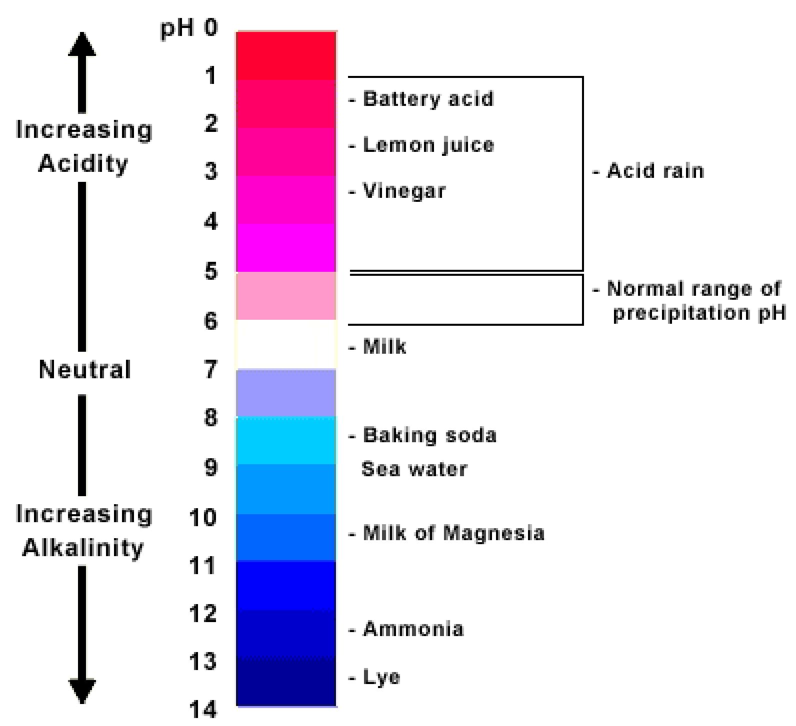
0.1M NaCl solution requires **0.1 x 58.44 g of NaCl** = **5.844g**
0.5M NaCl solution requires **0.5 x 58.44 g of NaCl** = **29.22g**
2M NaCl solution requires **2.0 x 58.44 g of NaCl** = **116.88g**

Understanding pH

The pH scale can tell if a liquid is more acidic or baseic. 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 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 X 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.

The pH Scale…



Acid-Base Indicators…

How else can pH be measured?
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.

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.

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.

Section 3

References

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

http://www.sciencecompany.com/lab/ph.htm