GOLDBIO®

User Guide for GoldBio Buffers

Procedure for use with Gold Biotechnology Buffers;

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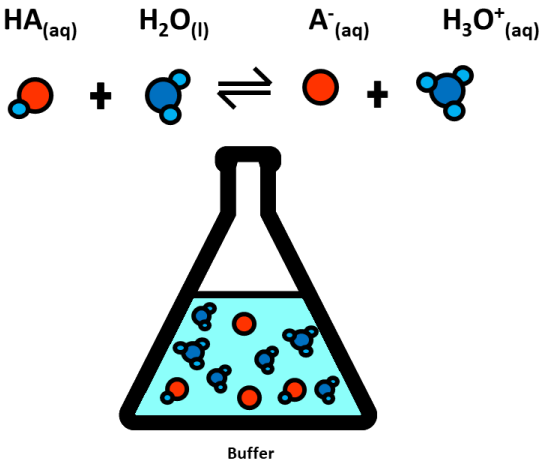
User Guide for GoldBio Buffers

Introduction

What is a buffer and how do they work?

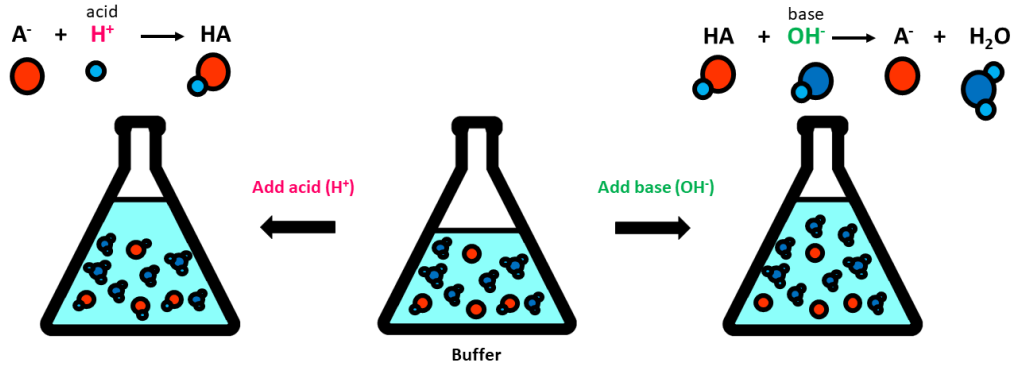
A buffering agent undergoes readily reversible proton exchange, picking up or dropping off hydrogen ions as they become available. A buffer solution can therefore maintain a stable pH upon the addition of an acid or base more easily than a simple solution of water. Another way to define a buffer is a solution comprised of a weak acid (proton donor, HA) and its conjugate base (proton acceptor, A-), or a weak base and its conjugate acid. For instance, a buffer solution can be made of acetic acid and sodium acetate. Such a buffer solution will be between pH 3-6 and will resist changes in pH upon addition of acid or base within that pH range.

HA can dissociate into A^- and H^+. H^+ then reacts with water to form H_3O^+. In the aqueous buffer solution, H_3O^+, HA and H^+ exist in equilibrium with each other. The buffering mechanism consists of two reversible reactions where the concentration of proton donor and proton acceptor are equal.



Buffer

Then, when a strong acid or base is introduced into this system by the scientist or by enzymatic activity during the experiment, the new ions from the introduced acid or base (H^+ or OH^-) are absorbed by the buffer and the pH remains stable preventing changes in protein structure and function.



Buffers cannot arbitrarily moderate any changes in ion concentration. Their optimal **buffering capacity, or range**, is defined by the dissociation constant, or ka, of the acid. We commonly discuss buffering capacity in terms of the pKa or the logarithmic constant of ka. We consider the buffering capacity of a specific buffer to be the pKa ± 1. For example, a buffer with a pH of 6.8 has a pH buffering range of 5.8-7.8.

Why is this important for biologists?

Since protonation alters the overall molecular charge, and can subsequently affect the structure of a molecule, most biological molecules are only functional in a certain pH range, and therefore, many organisms can only survive in a certain pH range. Researchers use buffers in the laboratory to maintain pH conditions that promote stable and functional molecules or organisms.

Why are there so many different buffers and how do I decide which one to use?

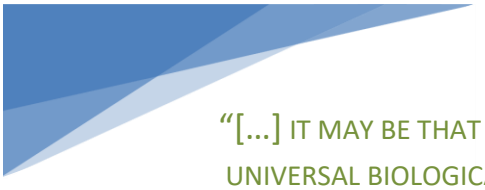
Buffers are chosen based upon the experiment that will be performed. For example, an Acetate buffer will work very well for maintaining a pH between 3 and 6, but it is of little use to you if you're studying infectious agents that spread through blood since blood usually maintains a pH around 7.4. While a phosphate buffer will provide the proper pH range, a phosphate buffer might not be appropriate if the experiment involves an enzyme that is affected by phosphate, such as many kinases, phosphatases, dehydrogenases, carboxypeptidases, ureases and more. Oftentimes, a zwitterionic buffer, such as one of Good's buffers, will work best. There are many different buffers, each with unique, physical properties that allow each individual buffer to have different conditions which are appropriate for use. Sometimes trying to decide which buffer to use can be difficult. Gold Biotechnology, Inc. has created this handbook to aid in the process of choosing a buffer so that you can spend *less* time looking and *more* time doing.

What are Good's Buffers?

Good's buffers are a set of biochemical buffers chosen by Norman Good based on a set of criteria essential to biological research. In total, there are 10 features that a buffer should

meet in order for it to be useful in research.

1. **pK_a is between 6 - 8:** Good's criteria were developed in order to identify buffers ideal for biochemical experiments. Therefore, the first criterion is that a biological buffer should be within a neutral pH range, matching biological systems – a pKa ranging between 6 and 8.
2. **Soluble in water:** Because biochemical reactions typically take place in aqueous environments, a buffer needs to be water-soluble.
3. **Membrane impermeability:** A biological buffer should not readily cross the cell membrane. Zwitterionic buffers such as MOPS and HEPES do not pass through the cell membrane.
4. **Minimal salt effects:** Since biological buffers are used in biological systems, high salt content could have disastrous effects on your sample. Therefore, buffers should have negligible amounts of ionic content.



"[...] IT MAY BE THAT THE QUEST FOR UNIVERSAL BIOLOGICAL INERTNESS IS FUTILE."
Norman E. Good

5. **Influences on dissociation:** Temperature, concentration and ionic strength can all have an impact on buffers in an experiment. However, for biochemical work, an ideal buffer would be one whose concentration, temperature and ionic composition of the medium will only have minimal effect on buffering capability (pKa).
6. **Well-behaved cation interactions:** The formation of complexes between a metal ion and the buffer results in proton release, which affects the pH of the system and may have an adverse effect on experimental results. Thus, these ionic complexes should be soluble and their binding constant must be known. A buffer with a low metal-binding constant is suitable for the study of metal-dependent enzymatic reactions. If your experimental design requires the use of a metal, then you should choose a buffer that does not form a complex with that specific metal.
7. **Stability:** Buffers should be stable and resist enzymatic and nonenzymatic degradation**.** And they should not interfere with enzyme substrates or resemble them.
8. **Biochemical inertness:** An ideal biological buffer will be inert, meaning that it won't influence or have an active role in biochemical reactions.
9. **Optical absorbance:** Because of the way biological buffers are used, they should not absorb light in the visible or ultraviolet regions of the spectrum. This criterion prevents interference in spectrophometric assays.
10. **Ease of Preparation and Affordability:** It is also important that a biological buffer be easy to use, from preparation to purification. And, they must be affordable.

From these criteria, Good *et al.* developed 20 buffers that are commonly referred to as "Good's buffers." Many other buffers have been developed to fit these criteria and are often referred to as "Good's buffers," despite not being developed by Good himself. No single buffer seems to fulfill all of these criteria, so selecting a buffer for one's own experiment often involves trial and error before selecting a suitable buffer. In the final analysis, the buffer must fulfill as many criteria as possible and sufficient controls should be designed to exclude unanticipated buffer effects.

The first property of a buffer that should be considered is the *pH range* required for the experiment. Every buffer has a pH range where the buffering capacity is highest, which is generally determined by the pK_a of the acid (or pK_as for polyprotic acids). **Choose a buffer that has pK_a that is at least within one pH unit of the target pH.** If studying a protein, the target pH will likely be a pH at which the protein is stable, which is generally at least one pH unit away from the pI of the protein.

Next, potential deleterious or unwanted buffer *interactions* with native molecules must be considered. Analyze the components of your system and determine, if possible, if there are any potential reactive species. Properly designed controls are critical to rule out buffer effects.

Table 1. List of Good's Buffers offered by GoldBio in order of pKa.

Buffer	pK_a at 20°C	pK_a /°C	Catalog Numbers
MES	6.15	-0.011	M-090, M-095, M-091
ADA	6.6	-0.011	A-780
PIPES	6.8	-0.0085	P-280, P-281
ACES	6.9	-0.020	A-010
MOPSO	6.95	-0.015	M-795, M-790
MOPS	7.15	-0.013	M-791, M-790
BES	7.15	-0.016	B-780
TES	7.5	-0.020	T-785
HEPES	7.55	-0.014	H-400, H-401
Tricine	8.15	-0.021	T-870
Tris	8.2		T-400
Bicine	8.35	-0.018	B-785
TAPS	8.55	-0.025	T-780
CHES	9.3		C-870
CAPS	10.4		C-040

How to Choose the Right GoldBio Buffer for Your Experiment

Questions to consider:

- *Are there metals in the solution?* Many buffers form a complex with certain metals. If this is the case with your preferred buffer, the concentration of the buffer may need to be adjusted and a stability constant for the buffer-metal complex should be taken into account. You might also want to consider the addition of a metal chelator, such as EDTA.

- *Are there carbohydrates, nucleotides or proteins in the solution?* Borate is known to form a complex with a wide variety of biological molecules. Most amine based buffers that have a neutral pH range, interact with DNA, which can have an effect on electrophoretic mobility and restriction enzyme kinetics.

- *Is your target protein an enzyme and if so, to what class of enzymes does it belong?* Phosphate and Tris, both widely used as buffers, can act as inhibitors for many different enzymes. Other buffers may have unintended, previously unknown effects as well.

- *What are the ionic strength requirements for ligand binding, protein stability or electrophoresis?* The ionic strength of the solution can affect the stability of some enzymes and the reaction kinetics with an enzyme substrate. The ionic strength of the solution will also affect electrophoretic mobility and conductivity during electrophoresis. This is important to keep in mind when preparing a buffer solution. A solution of "free acid" buffer that is brought to its pH with NaOH will have a different ionic strength than the sodium salt of that same buffer brought to the final pH with HCl due to the formation of NaCl in the latter example. The best way to avoid this is to combine the free acid buffer with the sodium salt of that buffer in molar proportions based on the Hendersen-Hasselbalch equation (see How to Make Your Buffer Solution). Mixing two buffers to achieve the desired pH allows the investigator to avoid the use of strong acids or bases.

- *Are there enzyme inhibitors in the solution?* Enzyme inhibitors are sometimes subject to interactions with buffers. DEPC, a popular RNase inhibitor, is inactivated by Tris and HEPES. However, DEPC is unaffected by MOPS or PBS. If Tris buffer must be used, treat the water with DEPC first, autoclave to destroy the DEPC and then Tris buffer may be used.

- *Does the study involve reduction-oxidation reactions?* HEPES and PIPES have demonstrated the ability to form radical species. Tricine in the presence of peroxide and enzymes with oxidase activity forms a stable nitric oxide-tricine radical species. MES, MOPS, HEPES and Tris have demonstrated the ability to slow Fe^{2+} autoxidation.

- *What sort of downstream assays and methods will be used?* Most zwitterionic buffers are known to interfere with the Lowry method of protein quantitation. Tris has been

observed to interfere with the Bradford assay. Some buffers have also been observed to interfere with protein quantitation using Bicinchoninic Acid (BCA). This interference with color development during the BCA assay can sometimes be overcome by precipitating the protein with deoxycholate and trichloroacetic acid or acetone. ADA absorbs UV light at 260 nm which can interfere with spectrophotometric nucleic acid quantitation.

- *Is there any literature regarding interactions of the buffer with any component of the system?* An effective literature search can sometimes go a long way in ruling out disruptive buffers for your experiment.

- Combining two buffers can provide advantages when compared to a single buffer system. While HEPES is a great buffer at physiological pH, the lowering of pH due to metabolic acidification in a cell culture can eventually bring the media out of the desired pH range and cause inhibition of cell growth. Bicarbonate/ CO_2 buffers alone are usually avoided because CO_2 can leave or enter the system freely, unintentionally changing the pH. Waldman *et al.* has shown that creating a buffering system that uses HEPES and bicarbonate can be more effective at maintaining a neutral extracellular pH of a cell culture than either buffer alone. Bicarbonate helps neutralize acid produced during metabolism while HEPES helps maintains the neutral pH after the addition of bicarbonate and after fluctuations of dissolved CO_2 levels.

The last thing to consider is the *concentration* of buffer you need to effectively maintain the pH. If the concentration of buffer is too low, it will not be effective. If the concentration is too high, there exists a greater chance that the buffer will interfere with the experiment. If the system being buffered does not actively exchange hydrogen protons, then a buffer concentration of 25-100mM is usually a good starting point. If proton exchange is likely to occur, then a buffer concentration that is 20X more than the molarity of the protons being exchanged should be used (**NOTE**: this is not always possible to calculate.) The buffer concentration also affects the ionic strength of the solution and hence, the electrophoretic mobility and conductivity during electrophoresis.

How to Make Your Buffer Solution

For instructions to make a specific buffer solution, see the protocols section at the end of this handbook.

A buffer solution is all about pH!

pH is a way to express the concentration of hydrogen protons in solution. This is relevant because the concentration of hydrogen protons will determine the ratio of protonated to deprotonated species of a biological molecule.

$pH = -\log[H^+]$

pK_a is the ionization constant; it is the pH at which a molecule becomes protonated or deprotonated, resulting in equal concentrations of the two chemical species. The buffering capacity is greatest, and therefore, a buffer works best to resist changes in pH, when the pH of a solution is at the pK_a value of a buffer. Each buffer should be used in a solution that is within one pH unit of the pK_a of the buffer. pH and pK_a can be related by the *Henderson-Hasselbalch* equation:

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

Wherein, **$[A^-]$** represents the concentration of the **conjugate base** and **[HA]** represents the **concentration of the acid**.

The *Henderson-Hasselbalch* equation can be used to determine the concentration of buffer acid and buffer salt needed to achieve the desired pH. For example, if you are trying to make a 1M solution of HEPES at pH 7.2 (25°C) you can insert the pK_a and the desired pH into the Henderson-Hasselbalch equation to find the ratio of HEPES free acid and HEPES sodium salt needed.
7.20 = 7.48 + log ([HEPES sodium salt]/[HEPES free acid]) This can also be expressed as follows:

[HEPES sodium salt]/[HEPES free acid] = 0.72

This ratio can be divided by two to determine the amount of sodium salt to use so that 0.36mol/L of HEPES sodium salt should be combined with 0.64mol/L of HEPES free acid to obtain a 1M solution with pH 7.2.

Zwitterionic free acids can also be made into a buffer solution using NaOH. Zwitterionic sodium salts can made into a buffer solution using HCl, but this will result in the formation NaCl as well.

Temperature Effects

It is important to know that the pK_a of a molecule and the pH of a solution changes when the temperature changes. d(pKa)/dT is an estimate of the change in pK_a with respect to temperature. The greater the absolute value of the d(pKa)/dT, the more the pK_a will change as the temperature changes. As the pK_a changes, the pH of the solution will change and the

buffering capacity will be greatest at the new pK_a. For some buffers the change is negligible and for others the change is great enough to consider using a different buffer if there will be dramatic temperature changes. For example, when using PIPES or ADA the pH drops 0.24 pH units when the temperature rises from 4°C to 37°C, but the pK_a and pH of a solution of Tris can change almost an entire pH unit as the temperature changes from 4°C to 37°C.

When preparing a buffer solution, it is important to make the solution at the temperature and concentration at which you intend to use it. Dissociation is dependent on temperature and concentration and can sometimes shift dramatically when one of these variables changes, affecting the pH of a solution. If diluting a stock solution of a buffer with high ionic strength, such as PIPES or ADA, be sure to check the pH of your working solution and adjust it if necessary. If the temperature of the media is going to fluctuate widely, be sure to choose a buffer than can accommodate these changes.

Buffers with the greatest temperature dependence:	
Tris	-0.028
Tricine	-0.021
TES	-0.020
ACES	-0.020
TAPS	+0.018
Bicine	-0.018

GoldBio Buffers Grouped By Structural Family

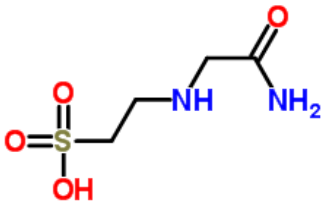
Most of the molecules deemed suitable as buffers for biological work can be grouped into six different structural families: Acetamido (ACES, ADA), Bis(2-hydroyethyl)amine (BES, Bicine, Bis-Tris), Cyclohexylamino (CAPS, CHES), Morpholinic (MES, MOPS, MOPSO), Piperazinic (HEPES, HEPPSO, PIPES), and Tris (Tris, TES, TAPS, Tricine, Bis-Tris).

Acetamido Family (ACES, ADA)

The Acetamido family includes ACES and ADA. Both of these buffering agents are known to complex with various metals. Despite the stronger acidity of the sulfonic acid group on ACES, the presence of the less substituted amine gives ACES a slightly higher pK_a than the diacetic amine substitution imparts to ADA.

ACES

pH range: 6.1 – 7.5



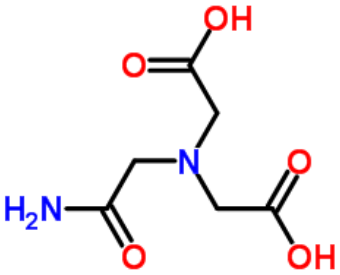
GoldBio Catalog: A-010
IUPAC Name: 2-[(2-Amino-2-oxoethyl)amino]ethanesulfonic acid
Formula: $C_4H_{10}N_2O_4S$
MW: 182.20 g/mol
pK_a at 25°C: 6.78
Solubility at 0°C: 0.22M
Metal complexation: strong complex with Mg^{2+} and Cu^{2+}; weak complex with other common metals

ACES is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. ACES is capable of forming complexes with some common metals, so formation constants should be taken into account when using this buffer in a solution containing metal ions. ACES absorbs UV light at 230 nm, which may interfere with some spectrophotometric assays. ACES is used as buffering component in cell culture media, in both capillary and gel electrophoresis (Liu *et al*. 1999, Hamoudová *et al*. 2006), and in various protein studies. It has been used as an enzyme assay buffer for the study of glucosidases (Hoskins *et al*. 1985), as a buffer for x-ray crystallography of aldehyde dehydrogenase enzyme complexes (Perez-Miller *et al*. 2003), and as a buffer solution to wash and heat *Lactobacillus plantarum* cells for the purpose of studying the effects of heat stress on the duration of the lag phase of growth of such cells (Smelt *et al*. 2002). It is used in the preparation of buffered charcoal yeast extract agar for the isolation of *Legionella* spp. (Edelstein *et al*. 1993), as well as in buffered culture media for hairy roots of *Catharanthus roseus (*Morgan *et al*. 2000). It has been observed to act as a competitive inhibitor of GABA receptor binding to rat brain synaptic membranes **and its use as a buffer should thus be avoided when studying GABA receptor interactions** (Tunnicliff *et al*. 1981).

ACES Keywords: *Legionella*, BCYE agar, x-ray crystallography, electrophoresis, *Catharanus roseus*, GABA inhibition, glucosidases

ADA

pH range: 6.0 – 7.2



GoldBio Catalog: A-780
IUPAC Name: 2,2'-[(2-Amino-2-oxoethyl)imino]diacetic acid
Formula: $C_6H_{10}N_2O_5$
MW: 190.22 g/mol
pK_a at 25°C: 6.59
Solubility at 0°C: solubility of free acid is very small; solubility of sodium salts very large
Metal complexation: strong complex with Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}; weak complex with other common metals

ADA is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. ADA is practically insoluble in water, but solubility of ADA increases if the free acid is converted to the sodium salt with NaOH (see ADA Stock Solution protocol). **ADA forms a strong complex with many common metals so stability constants should be taken into account when using this buffer**. While ADA can be used as a buffer in cation exchange chromatography, it should be used in lower concentrations because of the relatively large ionic strength and dependence of concentration on pK_a. ADA absorbs UV light between 0.1 and 260 nm, which may interfere with spectrophotometric assays.

ADA will not dissolve until you add a strong base!

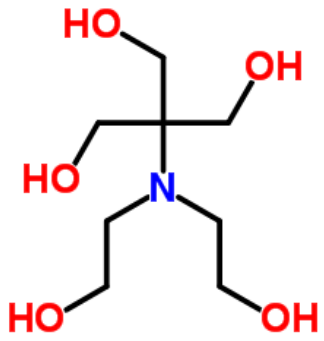
ADA buffer has been used successfully as a solution for protein crystallization (Wilson 2003, Dahl *et al*. 2007) and as a solution that permits microtubule polymerization to occur (Weisenberg *et al*. 1972). It was used as a running buffer during capillary electrophoresis and determined to provide the best resolution of the five buffers tested when separating amiodarone and desethylamiodarone (Pérez-Ruiz *et al*. 2002). ADA has been used as a sample buffer during differential scanning calorimetry to study FGF1, which could not be studied in phosphate or sulfate based buffers (Blaber *et al.* 1999). ADA has also been used as a complexing agent to effectively help remove contaminant metals from soil (Chen *et al* 1995). ADA is not suitable for use with the Bicinchoninic Acid (BCA) Assay.

ADA Keywords: crystallization, running buffer, capillary electrophoresis, FGF-1, differential scanning calorimetry

Bis(2-hydroxyethyl)amine Family (Bis-Tris, BES, Bicine)

The Bis(2-hydroyethyl)amine family includes Bis-Tris, BES, and Bicine. These buffering agents are known to complex with various metals. The substituted groups on the main moiety are, in order of increasing acidity: a carboxylic acid group (Bicine), a sulfonic acid group (BES), and a Tris(hydroxymethyl) group (Bis-Tris).

Bis-Tris



pH range: 5.8 – 7.2

GoldBio Catalog: B-020
IUPAC Name: 2-[Bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol
Formula: $C_8H_{19}NO_5$
MW: 209.24 g/mol
pK_a at 25°C: 6.46
Solubility at 0°C: >1M
Metal complexation: strong complex with Pb^{2+} and Cu^{2+}; weak complex with other common metals

Bis-Tris, also known as Bis-Tris Methane, is a zwitterionic buffering agent that contains functional groups related to two different structural families, the Bis(2-hydroxyethyl)amine family and the Tris family. Bis-Tris forms a strong complex with Cu^{2+} and Pb^{2+} and a weak complex with many common metals, so formation constants should be taken into account when using this buffer in a solution containing metal ions. Bis-Tris has been suggested as a substitute for the highly toxic buffer cacodylate (Scopes 1994).

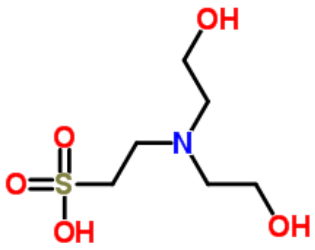
Try replacing cacodylate with a safer alternative like bis-tris for crystallization.

Bis-Tris buffered solutions are useful for resisting changes in pH during pressure changes (Funtenberger *et al*. 1995). Bis-Tris is used as a sample buffer, gel buffer and running buffer with various types of electrophoresis (Nijtmans *et al*. 2002, Klampfl *et al*. 1998) and as a buffer during anion exchange chromatography (Donoghue *et al*. 2000). It has also been used during x-ray crystallography of chloroplastic oxygen evolving enhancer protein (Kohoutova *et al*. 2009). Bis-Tris has been determined to be a useful buffer for NMR spectroscopy because of the low conductivity and high sensitivity that can be achieved using it (Kelly *et al*. 2002). Bis-Tris has been reported to interact with human liver fatty acid binding protein (FAB) and affect protein dynamics (Long *et al*. 2009). Bis-Tris is not suitable for use with the Bicinchoninic Acid (BCA) Assay.

Bis-Tris Keywords: electrophoresis, anion exchange chromatography, NMR spectroscopy, cacodylate

BES

pH range: 6.4 – 7.8



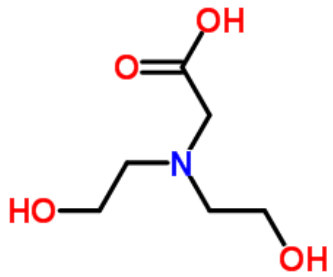
GoldBio Catalog: B-780
IUPAC Name: 2-[Bis(2-hydroxyethyl)amino]ethanesulfonic acid
Formula: $C_6H_{15}NO_5S$
MW: 213.25 g/mol
pK_a at 25°C: 7.09
Solubility at 0°C: 3.2M
Metal complexation: weak complex with Co^{2+} and Cu^{2+}

BES, like most neutral buffers that contain an amine group, forms a complex with DNA that affects restriction enzyme kinetics.

BES is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. BES can be used as a binding buffer and eluent in cation exchange chromatography as well as a buffer in gel filtration chromatography (Schirch *et al*. 1985). BES has been used in culture media as a buffer during bacteriophage adsorption (Lu *et al*. 2003). In an attempt to culture embryonic chondrocytes, it was found that using BES resulted in the adverse formation of cytoplasmic vacuoles, which were absent in the non-zwitterionic buffered media (Poole *et al*. 1982). BES forms a complex with DNA, affecting restriction enzyme kinetics. It interferes with reactions between DNA and restriction enzymes to a lesser extent than similar buffers with less substituted amine groups, such as Tris, but to a greater extent than HEPES (Wenner *et al*. 1999). BES can be used in a buffered saline solution in the calcium phosphate mediated transfection of eukaryotic cells with plasmid DNA (Chen *et al*. 1987, Schenborn 2000). Though BES binds to copper ions, it is still suitable for use with the Bicinchoninic Acid (BCA) Assay.

BES Keywords: cation exchange chromatography, gel filtration, bacteriophage adsorption, calcium phosphate transfection, BCA assay

Bicine



pH range: 7.6 – 9.0

GoldBio Catalog: B-785
IUPAC Name: N,N-Bis(2-hydroxyethyl)glycine
Formula: $C_6H_{13}NO_4$
MW: 163.17 g/mol
pK_a at 25°C: 8.26
Solubility at 0°C: 1.1M
Metal complexation: strong complex with Mg^{2+}, Ca^{2+}, Fe^{3+}, Co^{2+} and Cu^{2+}; weak complex with Mn^{2+}

Bicine is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. Bicine forms a strong complex with many common metals; therefore, stability constants and concentrations should be taken into consideration when choosing this buffer. It has been used in solutions for protein crystallization (Kanyo *et al*. 1992) and as a mobile phase buffer and eluent in cation exchange chromatography. Bicine can be used as a running buffer in doubled SDS-PAGE and has been reported to give better resolution and representation of proteins than the use of glycine or tricine (Williams *et al*. 2006). Bicine is commonly used as a buffer in the polymerase chain reaction, serving as both a pH buffer and a metal ion buffer (Innis *et al*. 1999). It has also been used in a multiphasic buffer system for SDS-PAGE of proteins (Wiltfang *et al* 1991). Bicine has been used as an extraction/grinding buffer for extraction of enzymes from various plant species, including salt-sensitive plants (Greenway *et al*. 1972, Yeoh *et al*. 1981). It was determined to be a suitable buffer for the culture of ammonia fungi at pH 8 (Yamanaka 2003). Bicine has been determined to be a useful buffer for NMR spectroscopy because of the low conductivity and high sensitivity that can be achieved using it (Kelly *et al*. 2002). Bicine is not suitable for use with the Bicinchoninic Acid (BCA) Assay.

For the best resolution during doubled SDS-PAGE, try using bicine.

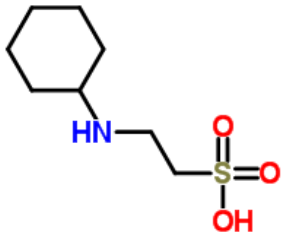
Bicine Keywords: crystallization, cation exchange chromatography, salt-sensitive plants, ammonia fungi, NMR spectroscopy, dSDS-PAGE, PCR

Cyclohexylamino Family (CHES, CAPS)

The Cyclohexylamino family includes CHES and CAPS. **Neither buffer is known to form any significant complex with metals**. They are used for studies occurring above physiological pH. The longer carbon chain of the sulfonic acid group in CAPS gives it a slightly more alkaline range than CHES.

CHES

pH range: 8.6 – 10.0



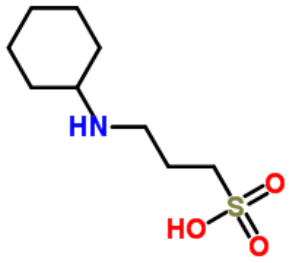
GoldBio Catalog: C-870
IUPAC Name: 2-(Cyclohexylamino)ethanesulfonic acid
Formula: $C_8H_{17}NO_3S$
MW: 207.29 g/mol
pK_a at 25°C: 9.5
Solubility at 0°C: 1.14M
Metal complexation: no significant complexes observed

CHES is a zwitterionic buffering agent used in biochemistry and molecular biology that is sometimes referred to as a Good's buffer despite not being introduced by Good *et al.* It is commonly used to study enzymatic processes above physiological pH. CHES displays only weak or negligible complexation capabilities for some metals making it a suitable noncoordinating buffer for use in most solutions that contain metal ions. It has been used as a buffer for enzymatic reactions (Omburo *et al*. 1992, Wasserman *et al*. 1984) and as a sample and running buffer for various types of capillary electrophoresis (Marina *et al*. 1996, Lavigne *et al*. 2007, Cunliffe *et al*. 2002). CHES displays high affinity for the iodoacetate binding site of liver alcohol dehydrogenase, protecting the enzyme from inactivation by iodoacetic acid (Syvertsen *et al*. 1981). Teresa Thiel *et al*. (1998) observed the greatest phosphodiesterase activity in CHES compared with the other buffers tested and showed that CHES can inhibit the viability of *Micrococcus luteus* at pH 9.5.

If your experiment requires alkaline conditions, consider using CHES or CAPS.

CHES Keywords: alkaline, capillary electrophoresis, phosphodiesterase, noncoordinating

CAPS



pH range: 9.7 – 11.1

GoldBio Catalog: C-040
IUPAC Name: 3-(Cyclohexylamino)-1-propanesulfonic acid
Formula: $C_9H_{19}NO_3S$
MW: 221.32 g/mol
pK_a at 25°C: 10.4
Solubility at 0°C: 0.47M
Metal complexation: no significant complexes observed

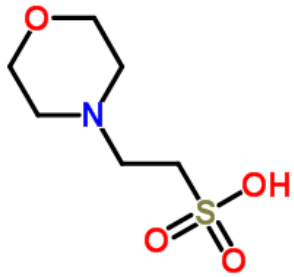
CAPS is another zwitterionic buffering agent used in biochemistry and molecular biology that was not introduced by Good *et al*., but is sometimes referred to as a Good's buffer. It is commonly used to study enzymatic processes above physiological pH. CAPS displays only weak or negligible complexation capabilities for some metals, making it a suitable noncoordinating buffer for use in solutions that contain metal ions. CAPS has been used as a transfer buffer for diffusion blotting and electroblotting (Thomas *et al*. 1996, Antonetti *et al*. 1999, Hulett *et al*. 1990), as a binding buffer and eluent in cation exchange chromatography (Thomas *et al*. 1996), as a running buffer in capillary electrophoresis (Prange *et al*. 1999), as an effective crystallization solution for various proteins (Zaitseva *et al*. 2004, Celie *et al*. 2005), and as an enzyme assay buffer (Hulett *et al*. 1990, Donarski *et al*. 1989, Jennings *et al*. 2001). CAPS can support alkaline phosphatase activity and inhibit the growth of *Aeromonas* spp. ATCC 31085 at pH 10.5 (Thiel *et al*. 1998). CAPS is suitable for use with the Bicinchoninic Acid (BCA) Assay.

CAPS Keywords: transfer buffer, Western blot, electroblotting, cation exchange, alkaline phosphatase, BCA

Morpholinic Family (MES, MOPS, MOPSO)

The Morpholinic family includes MES, MOPS, and MOPSO. These buffers are generally ideal for use in solutions that contain metal ions. The longer carbon chain of the sulfonic acid in MOPS makes it slightly less acidic than MES, while the additional hydroxyl group in MOPSO makes is slightly more acidic than MOPS.

MES



pH range: 5.5 – 6.7

GoldBio Catalog: M-090, M-095, M-091
IUPAC Name: 2-(4-Morpholinyl)ethanesulfonic acid
Formula: $C_6H_{13}NO_4S$
MW: 195.24 g/mol
pK_a at 25°C: 6.1
Solubility at 0°C: 0.65M
Metal complexation: possible complex with Fe^{3+}

MES is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. MES lacks the ability to form a complex with most metal ions and is recommended for use as a noncoordinating buffer in solutions with metal ions. MES is sometimes employed as a substitute for the highly toxic cacodylate, as well as a substitute for the ionic buffers citrate and malate. MES is often used in buffered culture media for bacteria, yeast and mammalian cells. It is toxic towards most plants at high concentrations, but can be used in plant culture media at concentrations of ~10mM (Parfitt *et al*. 1988).

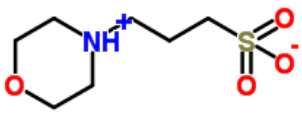
Looking for a nontoxic, zwitterionic alternative to cacodylate, citrate, or malate buffers? Try using MES.

Due to low ionic mobility and low conductivity at high concentrations, MES is regarded as an excellent buffer for use in capillary electrochromatography (Boughtflower *et al*. 1995). Those same properties make it **useful in many types of electrophoreses and chromatography** including gel-filtration chromatography (Weisenberg *et al*. 1995), phosphocellulose column chromatography (Alonso *et al*. 2000), hydrophobic interaction chromatography (Alonso *et al*. 2000), cation exchange chromatography (Cook *et al*. 1994), and SDS-PAGE (Kashino *et al*. 2001). MES has seen use been used for fluorescence microscopy (Neill *et al*. 2002), as an assay buffer to study Tau protein (Alonso *et al*. 2000), in a culture medium to initiate growth of pine and fir trees (Pullman *et al*. 2005), and in culture media for the growth of halophilic bacteria (Rodriguez-Valera *et al*. 1980). Heteromeric Cx32/Cx26 connexin channels isolated from mouse liver were seen to be inhibited by MES at any pH near or below its pK_a (Bevans *et al*. 1999). MES has been observed to complex with some organotin(IV) molecules that are being explored as antitumor agents (Mohamed *et al*. 2007) and to bind human liver fatty acid binding protein which affected the protein dynamics (Long *et al*. 2009).

MES Keywords: culture media, chromatography, running buffer, electrophoresis, fluorescence microscopy, Tau

MOPS

pH range: 6.5 – 7.9



GoldBio Catalog: M-790, M-791
IUPAC Name: 3-(Morpholin-4-ium-4-yl)-1-propanesulfonate
Formula: $C_7H_{15}NO_4S$
MW: 209.26 g/mol
pK_a at 25°C: 7.14
Solubility at 0°C: large
Metal complexation: possible complex with Fe^{3+}

MOPS is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. MOPS lacks the ability to form a complex with most metal ions and is recommended for use as a noncoordinating buffer in solutions with metal ions. MOPS is often used in cell culture media for bacteria, yeast and mammalian cells; though, concentrations higher than 20mM are not recommended for mammalian cell work. It is commonly used for cell culture media, as a running buffer in electrophoresis and for protein purification in chromatography.

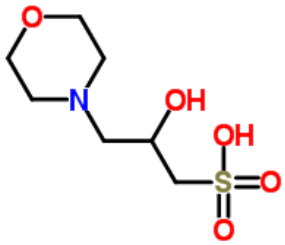
Do not autoclave MOPS! Sterilize by filtration to avoid degradation.

MOPS has been employed in cell culture media for the growth of enterobacteria (Neidhardt *et al.* 1974) and *Lactococcus lactis* (Jensen *et al*. 1993), in culture media to study meiotic regulation in mouse oocytes (Downs *et al*. 1997), and as an incubation buffer for MAP kinases isolated from rat cells (Zhang *et al*. 1997). In a study characterizing an atrazine metabolizing enzyme, de Souza *et al.* (1996) used it as a lysis buffer for *E. coli* cells, as an enzyme assay buffer for atrazine chlorohydrolase, as an eluent in gel filtration chromatography, and as a buffer during mass spectrometry. MOPS has been used to measure absorption during UV/VIS spectrophotometry and to study redox properties using cyclic voltammetry (Collins *et al*. 2005). MOPS is regarded as an excellent buffer for use in separating RNA in agarose gels, due to the high resolutions separation that occurs and a lack of interaction between MOPS and the RNase-inhibitor DEPC (Brody *et al*. 2004). It has also seen use as a running buffer in SDS-PAGE (Reichel *et al*. 2009). It is recommended to sterilize MOPS buffers by filtration rather than with autoclave due to the unknown identity of yellow degradation products that occur after sterilization of MOPS with autoclave. It is suitable for use in the Bicinchoninic Acid (BCA) Assay.

MOPS Keywords: culture media, running buffer, electrophoresis, chromatography, RNA, SDS-PAGE, BCA assay

MOPSO

pH range: 6.2 – 7.6



GoldBio Catalog: M-795, M-799
IUPAC Name: 2-Hydroxy-3-(4-morpholinyl)-1-propanesulfonic acid
Formula: $C_7H_{15}NO_5S$
MW: 225.26 g/mol
pK_a at 25°C: 6.87
Solubility at 0°C: 0.75M
Metal complexation: possible complex with Fe^{3+}

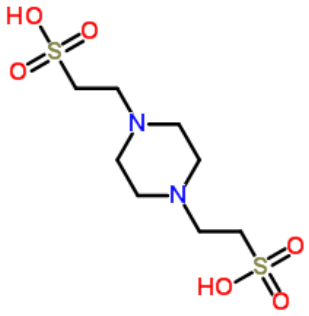
MOPSO is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. MOPSO lacks the ability to form a complex with most metal ions and is recommended for use as a noncoordinating buffer in solutions with metal ions. MOPSO has been used as a carrier electrolyte in capillary electrophoresis (Zemann *et al* 2001), as a grinding buffer for the extraction of sesquiterpene synthases from *Artemisia annua* (Bouwmmester *et al*. 1999), and as a crystallization buffer for homoglutathione synthetase (Galant *et al*. 2009). It has been used during fluorescence spectroscopy, spectrophotometry, and isothermal titration calorimetry (Ukaegbu *et al*. 2010). MOPSO has also been successfully used to maintain the pH in solutions of raw sugar, thereby preventing color fluctuations (Ahmedna *et al*. 1997). Colby *et al*. used it as a lysis buffer and enzyme assay buffer in a study characterizing spearmint limonene synthase from recombinant *E. coli* (1993). MOPSO interacts with the peptide backbone of bovine serum albumin (BSA) to stabilize BSA against thermal denaturation (Taha *et al* 2011).

MOPSO Keywords: capillary electrophoresis, crystallization, fluorescence spectroscopy, isothermal titration Calorimetry (ITC), bovine serum albumin

Piperazinic Family (PIPES, HEPES, HEPPSO)

The Piperazinic family includes PIPES, HEPES and HEPPSO. With the exception of a HEPPSO-Cu^{2+} complex, these buffers do not generally form complexes with metals. **They are generally not suitable for reduction-oxidation studies**. The ethanol group in place of one of the ethanesulfonic acid groups makes HEPES more alkaline than PIPES. The electron donating hydroxyl group present on the ethanesulfonic group of HEPPSO gives it a more alkaline nature than HEPES.

PIPES



pH range: 6.1 – 7.5

GoldBio Catalog: P-281, P-280
IUPAC Name: 2,2'-(1,4-Piperazinediyl)diethanesulfonic acid
Formula: $C_8H_{18}N_2O_6S_2$
MW: 302.37 g/mol
pK_a at 25°C: 6.76
Solubility at 0°C: solubility of free acid is very small; solubility of sodium salts very large
Metal complexation: no significant complexes observed

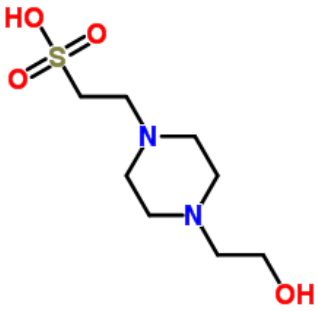
PIPES is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. **PIPES is practically insoluble in water, but solubility of PIPES increases if the free acid is converted to the sodium salt with NaOH** (see PIPES Stock Solution protocol). PIPES lacks the ability to form a significant complex with most metal ions and is recommended for use as a noncoordinating buffer in solutions with metal ions. This buffer is capable of forming radicals and is therefore not suitable for redox reactions (Baker *et al*. 2007). While **PIPES can be used as a buffer in cation exchange chromatography, it should be used in lower concentrations** because of the relatively large ionic strength and dependence of concentration on pK_a.

PIPES is an excellent buffer to use in place of cacodylate buffer for electron microscopy.

PIPES is regarded as an excellent buffer for the fixation of plant cells for electron microscopy (Bauer *et al*. 1977), and a suitable replacement for the highly toxic cacodylate buffer in this application (Yamamoto *et al*. 1983). It has been used to purify microtubule proteins using phosphocellulose chromatography (Clayton *et al*. 1980), in PHEM buffer alongside HEPES to study cytoskeletal components (Schliwa *et al*. 1981), to purify recombinant GTP-binding proteins, ARF1 and ARF2, by gel filtration (Cockcroft *et al*. 1994), and as a buffer solution to crystallize a transketolase enzyme from *E. coli* (Littlechild *et al*. 1995). PIPES has been observed to interact with a truncated variant of human bile salt-stimulate lipase (Moore *et al*. 2001) as well as inhibit a K^+-independent phosphatase reaction catalyzed by a (Na^+ + K^+) –ATPase from dog kidney (Robinson *et al*. 1987). It is suitable for use in the Bicinchoninic Acid (BCA) Assay.

PIPES Keywords: radicals, electron microscopy, plant cell fixation, microtubules, PHEM, crystallization, BCA assay, cell culture

HEPES



pH range: 6.8 – 8.2

GoldBio Catalog: H-400, H-401
IUPAC Name: 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid
Formula: $C_8H_{18}N_2O_4S$
MW: 238.30 g/mol
pK_a at 25°C: 7.48
Solubility at 0°C: 2.25M
Metal complexation: no significant complexes observed

HEPES is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. It meets many of Good's criteria for an effective buffer. It is useful at physiological pH, it does not readily complex with any metals, it is very soluble in water, it is relatively cheap, and it is biologically inert in most cases. An exception to its inertness is the participation in reduction-oxidation reactions to generate free radicals (Grady *et* al. 1988, Kirsch *et* al. 1998, Yang *et al.* 1999, Baker *et al*. 2007). HEPES is commonly used in cell culture media for various types of organisms and for protein studies that use it as a binding buffer and an eluent during cation exchange chromatography (Yan *et al*. 1998, Susin *et al*. 1999). It has been used to study pH effects on seed germination (Basto *et al.* 2013) and as a grinding buffer in plant studies (Soltis *et al*. 1983), as a running buffer (with or without Tris) in various forms of gel electrophoresis (Michels *et al.* 2002, Koort *et al*. 2002, Praveen 2009), and to flatten the pH gradient in polyacrylamide gels containing pH 5-7 ampholines during ultrathin-layer isoelectric focusing (Budowle 1987). It is not uncommon for HEPES to be used in DNA studies such as a buffer solution to form precipitates of calcium phosphate and DNA (Wigler *et al.* 1979), as a deposition buffer for atomic force microscopy (Lyubchenko *et al.* 2011), and as a buffer in electroporation (Fromm *et al.* 1985). HEPES interferes with reactions between DNA and restriction enzymes, but to a lesser extent than similar buffers with less substituted amine groups, such as Tris (Wenner *et al*. 1999). This lack of interference occurs mostly because steric hindrance of the protonated tertiary amine in HEPES allows for a weaker association with the negative charge of DNA than most

HEPES, like most neutral buffers that contain an amine group, forms a complex with DNA that affects restriction enzyme kinetics. Due to steric hindrance, this effect is minimal with HEPES when compared with Tris.

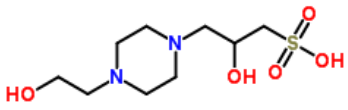
buffers, shielding DNA from restriction enzymes to a lesser extent than less substituted buffers.

Itagaki and Kimura (1979) demonstrated that HEPES can be used to buffer mammalian cell cultures in closed systems of high and low density cultures, as well as open systems of high density cultures. Zigler *et al.* (1985) showed that HEPES was capable of increasing the phototoxicity of mammalian tissue culture media containing riboflavin in a study done with murine thymocytes. This phototoxicity is attributed to the inhibition of thymidine uptake caused by the generation of hydrogen peroxide. Photoreactivity may be decreased by keeping HEPES-buffered media in the dark or by lowering or eliminating the amount of riboflavin, tryptophan and tyrosine present in media (Stoien *et al*. 1974). A fair amount of studies have demonstrated the usefulness of HEPES as buffer for *in vitro* fertilization and embryo culture (Mahadevan *et al*. 1986, Behr *et al.* 1990)). **Heteromeric Cx32/Cx26 connexin channels isolated from mouse liver were seen to be inhibited by HEPES at any pH near or below its pK_a, indicating that HEPES is a poor buffer to use for studying such heteromeric connexin channels** (Bevans *et al*. 1999). The research of Habib and Tabata (2004) led to an understanding of the free radicals generated by HEPES when $Au3^+$ was present. Their research indicated that the damage to DNA was caused by nitrogen-centered cationic free radicals and not reactive oxygen species (ROS). Supporting the addition of HEPES to Tris-based buffers, Ahmad and Ghasemi (2007) showed that **adding HEPES to the traditional Tris buffer used in quantitative real-time PCR provided lower background levels and expanded dynamic range**. HEPES has been deemed suitable for use as a buffer in quantitative protein measurement with the Bicinchoninic Acid assay (BCA) (Kaushal *et al*. 1986), but unsuitable for use with the Lowry (Folin) method of protein quantitation (Peterson 1979).

HEPES Keywords: cation exchange, running buffer, electrophoresis, chromatography, DNA, electroporation, phototoxicity with riboflavin, *in vitro* fertilization (IVF), quantitative real time polymerase chain reaction (qPCR), restriction enzymes, atomic force microscopy, seed germination, radicals

HEPPSO

pH range: 7.1 – 8.5



GoldBio Catalog: H-480
IUPAC Name: 2-Hydroxy-3-[4-(2-hydroxyethyl)-1-piperazinyl]-1-propanesulfonic acid
Formula: $C_9H_{20}N_2O_5S$
MW: 268.33 g/mol
pK_a at 25°C: 7.84
Solubility at 0°C: 2.20M
Metal complexation: weak complex with Cu^{2+}

HEPPSO is a zwitterionic buffer that was selected and described by Good *et al.* in 1966. HEPPSO has been used as a crystallization buffer solution for glycogen synthase enzymes and for enzymes complexed with HEPPSO (Sheng *et al* 2009). HEPPSO displayed little to no toxicity for the indicator species *Daphnia magna* and has thus been deemed suitable for toxicological studies (Keating *et al*. 1996). This buffer is capable of forming radicals and is therefore not suitable for redox reactions. HEPPSO forms a complex with Cu^{2+} ions; therefore, stability constants and concentrations should be taken into consideration when using this buffer in a solution containing copper ions. Though HEPPSO binds to coppers ions, it is still suitable for use with the Bicinchoninic Acid (BCA) Assay (Kaushal *et al*. 1986).

HEPPSO is suitable for toxicity studies.

HEPPSO Keywords: crystallization, glycogen synthase, nontoxic, BCA assay, radicals

Tris Family (Tris, Bis-Tris, TES, TAPS, Tricine)

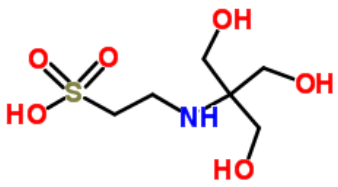
The Tris family includes Tris, Bis-Tris, TES, TAPS, and tricine. These buffers are known to form complexes with various metals. They are widely used in electrophoresis.

Bis-Tris

See description under Bis(2-hydroxyethyl)amine Family heading.

TES

pH range: 6.8 – 8.2



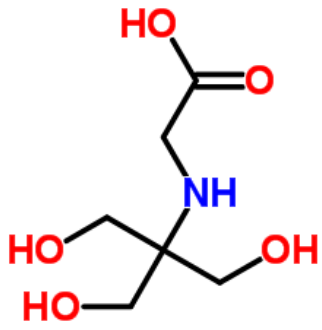
GoldBio Catalog: T-785
IUPAC Name: 2-{[1,3-Dihydroxy-2-(hydroxymethyl)-2-propanyl]amino}ethanesulfonic acid
Formula: $C_6H_{15}NO_6S$
MW: 229.25 g/mol
pK_a at 25°C: 7.40
Solubility at 0°C: 2.60M
Metal complexation: strong complex with Cr^{3+} and Fe^{3+}; weak complex with Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}

TES is a zwitterionic buffering agent used in biochemistry and molecular biology that was selected and described by Good *et al.* **It has a pK_a that is right at physiological pH (7.4) at 25°C**, making it a useful buffer for many biologists. Comai & Kosuge (1980) used TES as a cell wash buffer and as a solution from which to precipitate and extract DNA. TES has also been used to help purify microsomes from porcine kidney by gel filtration and affinity chromatography, and was used in the same study as an enzyme assay buffer (Mumford *et al*. 1981). TES is used in Test Yolk Buffer, which is known as a first choice solution for preserving spermatozoa (Weidel *et al*. 1987, Hammadeh *et al*. 2001). Like most neutral pH amine-based buffers, TES forms a complex with DNA; it affects restriction enzymes kinetics more than HEPES but less than Tris (Wenner *et al* 1999). TES has been used in media for viral plaque assays (Richter 1967). It is suitable for use in the Bicinchoninic Acid (BCA) Assay.

TES Keywords: physiological pH, affinity chromatography, gel filtration, Test Yolk Buffer, viral plaque assay, BCA assay

Tricine

pH range: 7.4 – 8.8



GoldBio Catalog: T-870
IUPAC Name: N-[1,3-Dihydroxy-2-(hydroxymethyl)-2-propanyl]glycine
Formula: $C_6H_{13}NO_5$
MW: 179.17 g/mol
pK_a at 25°C: 8.05

Solubility at 0°C: 0.8M
Metal complexation: strong complex with Mg^{2+}, Ca^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+}

Tricine is a zwitterionic buffering agent used in biochemistry and molecular biology that was selected and described by Good *et al.* Tricine is commonly used instead of glycine in the Laemmli buffer during SDS-PAGE to separate small peptides (Schägger 1987). It is used in animal tissue culture (Gardner 1969) and helps inhibit the unintended growth of mycoplasma (Spendlove *et al*. 1971). Tricine has also been added to bacterial culture media to prevent precipitation of iron salts (Neidhardt *et al*. 1974). Tricine was determined to allow more light production from firefly luciferase in the presence of ATP than other buffers tested (Webster *et al*. 1980). Tricine has been used as an enzyme assay buffer for isocitrate dehydrogenase and glutamate dehydrogenase (Gambord *et al*. 1970).

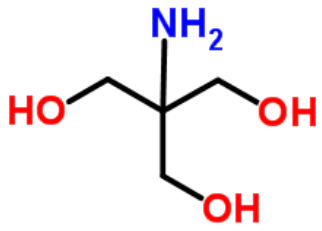
Try using tricine in place of the controlled substance barbital when following protocols that use barbital as a buffer.

Tricine can bind hydroxyl radicals, forming formaldehyde in the process (Shiraishi *et al*. 1993, Hicks *et al*. 1986). It can also form tricine nitroxide radicals; this should be taken into consideration if using tricine in the presence of an enzyme with oxidase activity (Grande *et al.* 1978). **Tricine forms a strong complex with many common metals so stability constants and concentrations should be taken into consideration when choosing this buffer**. Even though tricine forms a complex with Cu^{2+}, the addition of Cu^{2+} to a solution enables tricine to be used in the Lowry protein assay. Tricine is not suitable for use with the Bicinchoninic Acid (BCA) Assay. Tricine has been used as a substitute for protocols that utilize the controlled substance barbital as a buffer for electrophoresis (Monthony *et al.* 1978).

Tricine Keywords: Laemmli buffer, SDS-PAGE, animal tissue culture, bacterial culture media, firefly luciferase, isocitrate dehydrogenase, glutamate dehydrogenase, radicals, Lowry protein assay, barbital

Tris

pH range: 7.0 – 9.0



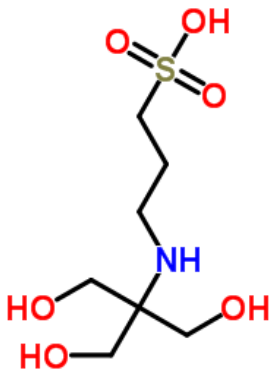
GoldBio Catalog: T-400, T-095, T-090
IUPAC Name: 2-Amino-2-(hydroxymethyl)-1,3-propanediol
Formula: $C_4H_{11}NO_3$
MW: 121.14 g/mol
pK_a at 25°C: 8.06
Solubility at 0°C: 2.40M
Metal complexation: strong complex with Cr^{3+}, Fe^{3+}, Ni^{2+}, Co^{2+}, and Cu^{2+}; weak complex with other common metals

Short for Tris (hydroxymethyl) aminomethane (THAM), Tris is an organic compound often used in buffer solutions for gel electrophoresis, such as TAE or TBE. It is used in the preparation of Laemmli buffer, one of the most common running buffers for SDS-PAGE. In addition, Tris can also be used for many custom running and loading buffers, most often with glycine and SDS. It can be used as a buffer and eluent in anion exchange chromatography (Huisman *et al*. 1965). The use of **single-junction pH electrodes that contain silver should be avoided** when determining the pH of a solution containing Tris buffer (Benesch *et al*. 1955). **The pH of a solution containing Tris is very dependent on temperature; while temperature and concentration should be taken into to consideration for every buffer, it is especially important to prepare Tris buffer at the temperature at which it will be used** (El-Harakany *et al*. 1984, Vega *et al*. 1985). The primary amine group of Tris reacts, to some extent, with a variety of molecules, including the RNAse inhibitor DEPC, many aldehydes, common metals, many enzymes and DNA. This reactivity combined with the tendency of Tris to permeate membranes **excludes it from most cell culture work**. Tris is not suitable for use with the Bicinchoninic acid (BCA) assay.

Do not use single-junction pH electrodes in Tris buffer.

Tris Keywords: running buffers, electrophoresis, TAE, TBE, Laemlli, glycine, SDS-PAGE, anion exchange chromatography, temperature dependent

TAPS



pH range: 7.7 – 9.1

GoldBio Catalog: T-780
IUPAC Name: 3-{[1,3-Dihydroxy-2-(hydroxymethyl)-2-propanyl]amino}-1-propanesulfonic acid
Formula: $C_7H_{17}NO_6S$
MW: 243.28 g/mol
pK_a at 25°C: 8.40
Solubility at 0°C: large
Metal complexation: strong complex with Cr^{3+}, Fe^{3+}, and Cu^{2+}; weak complex with other common metals

TAPS is a zwitterionic buffering agent used in biochemistry and molecular biology that was selected and described by Good *et al.* TAPS has been used in planar chromatography to separate dyes (Nurok *et al*. 2000), in ion exchange chromatography to purify glutathione S-transferase isolated from atrazine-resistant *Abutilon theophrasti* (Anderson *et al*. 1991), and as an enzyme assay buffer for aminopeptidase activity (Nishiwaki *et al*. 2000). It is a preferred culture media buffer to use for dinoflagellate experiments, allowing for minimal pH change and maximal growth (Loeblich *et al*. 1975). Heteromeric Cx32/Cx26 connexin channels isolated from mouse liver were seen to be inhibited by TAPS at any pH near or below its pK_a (Bevans *et al*. 1999). TAPS forms a strong complex with Cr^{3+}, Fe^{3+}, and Cu^{2+} and a weak complex with some other common metals, so stability constants and concentrations should be taken into account when choosing this buffer.

TAPS Keywords: planar chromatography, ion exchange chromatography, glutathione S-transferase, dinoflagellate, aminopeptidase, connexin inhibition

Other buffers

PBS (Phosphate Buffered Saline)

pH range: 7.2 – 7.4

GoldBio Catalog: P-271

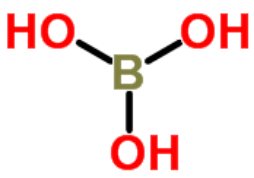
PBS (phosphate buffered saline) is a common buffer solution used in the laboratory. PBS buffer tablets from GoldBio make a solution that contains 10mM phosphate buffer, 137mM sodium chloride, and 2.7mM potassium chloride. It is an isotonic and nontoxic

buffer that is meant to mimic the pH, osmolarity, and ion concentrations of the human body, making it particularly useful in tissue cultures at physiological pH (7.4). PBS is used for immunoassays, for sample dilution, for protein purification, and as a wash buffer in cell cultures. The addition of EDTA to PBS allows the solution to be used to disengage attached or clumped cells because of the chelation of Ca^{2+} and Mg^{2+} ions involved in adhesion.

PBS Keywords: isotonic, physiological pH, immunoassays, sample dilution, cell culture

Boric Acid

pH range: 8.2 – 10.1



GoldBio Catalog: B-030, B-031
IUPAC Name: Boric acid
Formula: H_3BO_3
MW: 61.83 g/mol
pK_a at 25°C: 9.23
Solubility at 0°C: 0.4M

Boric acid is commonly used for the preparation of buffers such as Tris-Borate-EDTA (TBE). Borate buffers have been used in capillary and gel electrophoresis for separating proteins, nucleic acids, and secondary metabolites such as catecholamines (Kaneta *et al* 1991). The useful buffering range of boric acid buffers is pH 8.2 - 10.1. Boric acid and its related salts form a complex equilibria of at least 10 reactions, and at pH 8 or higher, the tetraborate ion ($B_4O_7^{2-}$) is the most predominant species present (Salentine, *et* al. 1983, Trejo *et al*. 2012). Boric acid is used in catalytic amounts to promote amidation between carboxylic acids and amines (Tang 2005). It is capable of forming a complex with various carbohydrates (Aronoff *et al*. 1975), as well as with pyridine nucleotides, such as NAD^+ (Johnson *et al*. 1976). Boron is also an essential micronutrient for plants and can be supplied in the form of boric acid (Warington 1923).

Boric Acid Keywords: TBE, electrophoresis, plant micronutrient, amidation

FAQS

Why are the solubilities given at 0°C if I'm going to be making my buffer at room temperature?

Norman Good determined the solubility of all the buffers with which he worked at 0°C. This is important to know if the buffer will be used in an ice bath or cold room so that precipitation of the buffer can be avoided.

Why won't ADA or PIPES go into solution?

Both ADA and PIPES have very limited solubility in their free acid forms. Once conditions become less acidic, usually through the addition of NaOH, these buffers will go into solution.

It doesn't look like Bicine or Tricine is going into solution. What do I do?

Add the full volume of water and mix well to make Bicine or Tricine at the concentration suggested in each protocol. **NOTE**: A lower concentration should be used if the solution is to be frozen.

What's the difference between Tris-HCl and Tris base?

Tris HCl is used to simplify the process of making Tris buffer solutions. It also allows for more reproducibility. Rather than adding HCl or NaOH to adjust the pH, you can use Tris-HCl instead. You can find more details in this article.

My buffer solutions look a little cloudy. Is this okay?

Is the buffer in question MOPS? MOPS will sometimes have a light yellow appearance. Swirl the bottle to see if anything settles at the bottom or if the solution remains consistent. If it is dark yellow or if the solution is not consistent, dispose of the solution properly. For other buffers, a cloudy appearance is likely occurring due to contamination of microbes. It is also possible that your buffer solution has become chemically unstable. Dispose of the solution properly and prepare new buffer solutions.

How do I make an RNAse-free solution containing Tris or HEPES?

The reactive amine group on Tris reacts with diethylpyrocarbonate (DEPC); therefore, when doing RNA work be sure you do not treat Tris-containing solutions with DEPC. HEPES also interferes with the inactivation of RNAses by DEPC. You can treat a solution of deionized water with DEPC to inactivate any RNAses, autoclave the solution to inactivate the DEPC, and then add the buffer. Instead, consider using GoldBio MOPS for RNA work.

How do I choose a buffer in which my protein will be soluble and active?

Make sure that the pH for which you're aiming is far away from the isoelectric point (pI) of your protein. Then, read to find out what other researchers before you have used on similar proteins. Try experimenting with different buffers that are in the proper pH range. Ionic strength is very important so try changing the concentration of salt in your buffer solution. Determine if there is a metal needed that is critical for protein structure. Determine if there is any metal in solution that might disrupt the protein structure and if so, try adding a metal chelator like EDTA.

Troubleshooting

Why are my cells dying in HEPES-buffered media?
If your media contains riboflavin, then HEPES is most likely increasing the phototoxicity of the media to your cells. Try lowering the amount of riboflavin, tyrosine or tryptophan in the media, or try protecting the culture from light by wrapping it in foil.

I've used a certain buffer to grow these same embryos before and seen great expression of gene A but now I'm seeing poor expression of gene B. Could my choice of buffer be affecting this observation?
Gene expression of embryos or oocytes grown *in vitro* can actually be affected by buffer choice (Palasz *et al* 2008).

Protocols

Protocols to make a buffer solution using each of the GoldBio buffering agents are given in alphabetical order.

Stock Solutions:

ACES
ADA
BES
Bicine
Bis-Tris
CAPS
CHES
HEPES
HEPES, Sodium
HEPPSO
MES
MES, Monohydrate
MES, Sodium
MOPS
MOPS, Sodium
MOPSO
MOPSO, Sodium
PIPES
PIPES, Sodium
TAPS
TES
Tricine
Tris
Tris HCl

0.1M ACES Buffer - 1 L

Instructions

1. Dissolve 18.22 g of ACES (ACES, GoldBio Catalog # A-010 [CAS 7365-82-4, mw. = 182.20 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 0.1M ACES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 4.09
Adjust pH with: 10N NaOH

pH	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5
mL	1.1	1.2	1.42	1.72	2.02	2.5	2.9	3.3	3.75	4.25	4.71	5.11	5.49	5.89	6.21

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

ACES pKa at 25°C: 6.78
ACES pH range: 6.1 -7.2
d(pKa)/dT value: -0.02

0.5M ADA Buffer - 1 L

Instructions

1. Suspend 95.11 g of ADA (ADA, GoldBio Catalog # A-780 [CAS 26239-55-4, mw. = 190.22]) in 750 mL of dH_2O. ADA will not readily dissolve until the pH is raised.

2. Adjust to desired pH using 10N NaOH.

3. Fill to final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at 4°C.

To make a 1 L solution of 0.5M ADA, use the table below to estimate the required volume of base for a given pH:

Starting pH: 1.95
Adjust pH with: 10N NaOH

pH	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2
mL	6.3	6.5	6.75	7	7.25	7.5	7.8	8.1	8.35	8.5	8.7	8.9	9.05

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

ADA pKa at 25°C: 6.59
ADA pH range: 6.0 – 7.2
d(pKa)/dT value: -0.011

1M BES Buffer - 1 L

Instructions

1. Dissolve 213.25 g of BES (BES, GoldBio Catalog # B-780 [CAS 10191-18-1, mw. = 213.25 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 1M BES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 2.55
Adjust pH with: 10N NaOH

pH	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8
mL	11	12.5	15	19	22	27	32	38	43	50	56.5	63	71	76	82

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

BES pKa at 25°C: 7.09
BES pH range: 6.4 – 7.8
d(pKa)/dT value: -0.016

1M Bicine Buffer - 1 L

Instructions

1. Dissolve 163.17 g of Bicine (Bicine, GoldBio Catalog # B-785 [CAS 150-25-4, mw. = 163.17 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 1M Bicine, use the table below to estimate the required volume of base for a given pH:

Starting pH: 5.30
Adjust pH with: 10N NaOH

pH	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
mL	9	11	12	17	21	26	31	36	40	46	52	58	63	69	74

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

Bicine pKa at 25°C: 8.26
Bicine pH range: 7.6 – 9.0
d(pKa)/dT value: -0.018

1M Bis-Tris Buffer - 1 L

Instructions

1. Dissolve 209.24 g of Bis-Tris (Bis-Tris, GoldBio Catalog # B-020 [CAS 6976-37-0, mw. = 209.24 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using concentrated HCl.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 1M Bis-Tris, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 9.90
Adjust pH with: conc. HCl

pH	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2
mL	77	76	74	72	70	67	64	61	57	53	49	44	39	35	30	26	22	18

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

Bis-Tris pKa at 25°C: 6.50
Bis-Tris pH range: 5.8 – 7.2
d(pKa)/dT value: -0.017

0.5M CAPS Buffer - 1 L

Instructions

1. Dissolve 110.66 g of CAPS (CAPS, GoldBio Catalog # C-040 [CAS 1135-40-6, mw. = 221.32 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 0.5M CAPS, use the table below to estimate the required volume of base for a given pH:

Starting pH: 5.03
Adjust pH with: 10N NaOH

pH	9.7	9.8	9.9	10.0	10.1	10.2	10.3	10.4	10.5	10.6	10.7	10.8	10.9	11.0	11.1
mL	6	8	9	11	13	16	18	21	23.5	26	29	32	34	37	39

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

CAPS pKa at 25°C: 10.4
CAPS pH range: 9.7 – 11.1
d(pKa)/dT value: -0.009

0.5M CHES Buffer - 1 L

Instructions

1. Dissolve 103.65 g of CHES (CHES, GoldBio Catalog # C-870 [CAS 103-47-9, mw. = 207.29 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 0.5M CHES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 4.13
Adjust pH with: 10N NaOH

pH	8.6	8.7	8.8	8.9	9.0	9.1	9.2	9.3	9.4	9.5	9.6	9.7	9.8	9.9	10.0
mL	5	7	11	15	18	20	22	24	27	30	33	35	37	39	41

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

CHES pKa at 25°C: 9.5
CHES pH range: 8.6 – 10.0
d(pKa)/dT value: -0.011

1M HEPES Buffer - 1 L

Instructions

1. Dissolve 238.30 g of HEPES (HEPES, Free Acid, GoldBio Catalog # H-400 [CAS 7365-45-9, mw. = 238.30]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of HEPES free acid and HEPES sodium salt (HEPES, Sodium Salt, GoldBio Catalog # H-401 [CAS 75277-39-3, mw. = 260.29]) can be mixed to attain a pH of ~7.5. The pH can be adjusted by increasing the molar ratio of HEPES free acid (more acidic) or HEPES sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M HEPES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 5.63
Adjust pH with: 10N NaOH

pH	6.8	6.9	7	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8	8.1	8.2
mL	6	8	10	14	17	20	24	28	33	38	44	49	55	61	67

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

HEPES pKa at 25°C: 7.50
HEPES pH range: 6.8 – 8.2
d(pKa)/dT value: -0.014

1M HEPES-Na Buffer - 1 L

Instructions

1. Dissolve 260.29 g of HEPES, Sodium Salt (HEPES, Sodium Salt, GoldBio Catalog # H-401 [CAS 75277-39-3, mw. = 260.29]) in 750 mL of dH_2O.

2. Adjust to desired pH using concentrated HCl. Note: This method will produce about 0.25M – 0.9M NaCl in the concentrated stock solution.

3. Fill to final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at 4°C.

Note: Alternatively, equimolar concentrations of HEPES free acid (HEPES, Free Acid, GoldBio Catalog # H-400 [CAS 7365-45-9, mw. = 238.30]) and HEPES sodium salt can be mixed to attain a pH of ~7.5. The pH can be adjusted by increasing the molar ratio of HEPES free acid (more acidic) or HEPES sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M HEPES, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 10.56
Adjust pH with: conc. HCl

pH	6.8	6.9	7	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8	8.1	8.2	8.3
mL	75	73	71	68	65	62	59	55	50	46	43	37	32	28	24	21

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

HEPES pKa at 25°C: 7.50
HEPES pH range: 6.8 – 8.2
d(pKa)/dT value: -0.014

1M HEPPSO Buffer - 1 L

Instructions

1. Dissolve 268.33 g of HEPPSO (HEPPSO, GoldBio Catalog # H-480 [CAS 68399-78-0, mw. = 268.33]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 1M HEPPSO, use the table below to estimate the required volume of base for a given pH:

Starting pH: 5.89
Adjust pH with: 10N NaOH

pH	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8	8.1	8.2	8.3	8.4	8.5
mL	3.9	5.0	6.4	8.2	10.7	13.3	16.2	19.5	24.3	28.3	33.5	38.8	43.3	49.0	56.0

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

HEPPSO pKa at 25°C: 7.85
HEPPSO pH range: 7.1 – 8.5
d(pKa)/dT value: -0.010

0.5M MES Buffer - 1 L

Instructions

1. Suspend 97.62 g of MES free acid (MES, Free Acid, GoldBio Catalog # M-095 [CAS 4432-31-9, mw. = 195.24 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of MES free acid and MES sodium salt (MES, Sodium Salt, GoldBio Catalog # M-091 [CAS 71119-23-8, mw. = 217.22]) can be mixed to attain a pH of ~ 6.1. The pH can be adjusted by increasing the molar ratio of MES free acid (more acidic) or MES sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 0.5M MES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 3.23
Adjust pH with: 10N NaOH

pH	5.5	5.6	5.7	5.8	5.9	6	6.1	6.2	6.3	6.4	6.5	6.6	6.7
mL	5	6.2	7.6	9.3	11.4	13.6	15.8	18.3	21.1	23.7	26.4	28.7	31.1

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

MES pKa at 25°C: 6.10
MES pH range: 5.5 – 6.7
d(pKa)/dT value: -0.011

0.5M MES Monohydrate Buffer - 1 L

Instructions

1. Suspend 106.53 g of MES free acid monohydrate (MES, Free Acid Monohydrate, GoldBio Catalog # M-090 [CAS 145224-94-8, mw. = 213.25 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of MES free acid monohydrate and MES sodium salt (MES, Sodium Salt, GoldBio Catalog # M-091 [CAS 71119-23-8, mw. = 217.22]) can be mixed to attain a pH of ~ 6.1. The pH can be adjusted by increasing the molar ratio of MES free acid (more acidic) or MES sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 0.5M MES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 2.92
Adjust pH with: 10N NaOH

pH	5.5	5.6	5.7	5.8	5.9	6	6.1	6.2	6.3	6.4	6.5	6.6	6.7
mL	5	6.1	7.4	8.7	10.8	12.6	14.6	16.8	19.2	21.7	24	26.3	28.2

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

MES pKa at 25°C: 6.10
MES pH range: 5.5 – 6.7
d(pKa)/dT value: -0.011

0.5M MES-Na Buffer - 1 L

Instructions

1. Suspend 108.61 g of MES sodium salt (MES, Sodium Salt, GoldBio Catalog # M-091 [CAS 71119-23-8, mw. = 217.22]) in 750 mL of dH_2O.
2. Adjust to desired pH using concentrated HCl. This method will produce 0.12M -0.38M NaCl in the concentrated stock solution.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of MES sodium salt and MES free acid (MES, Free Acid, GoldBio Catalog # M-095 [CAS 4432-31-9, mw. = 195.24 g/mol]) can be mixed to attain a pH of ~ 6.1. The pH can be adjusted by increasing the molar ratio of MES free acid (more acidic) or MES sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 0.5M MES, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 10.08
Adjust pH with: conc. HCl

pH	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8
mL	32	31	30	29	28	26	24	22	20	18	16	14	12	10

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

MES pKa at 25°C: 6.10
MES pH range: 5.5 – 6.7
d(pKa)/dT value: -0.011

1M MOPS Buffer - 1 L

Instructions

1. Dissolve 209.26 g of MOPS free acid (MOPS Free Acid, Ultra Pure, GoldBio Catalog # M-790 [CAS 1132-61-2, mw. = 209.26 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of MOPS free acid and MOPS sodium salt (MOPS, Sodium Salt, GoldBio Catalog # M-791 [CAS 71119-22-7, mw. = 231.25]) can be mixed to attain a pH of ~ 7.2. The pH can be adjusted by increasing the molar ratio of MOPS free acid (more acidic) or MOPS sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M MOPS, use the table below to estimate the required volume of base for a given pH:

Starting pH: 3.82
Adjust pH with: 10N NaOH

pH	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9
mL	7	9	11	13	16	20	24	28	33	39	45	51	57	62	68

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

MOPS pKa at 25°C: 7.14
MOPS pH range: 6.5 – 7.9
d(pKa)/dT value: -0.011

1M MOPS-Na Buffer - 1 L

Instructions

1. Dissolve 231.25 g of MOPS, Sodium Salt (MOPS, Sodium Salt, GoldBio Catalog # M-791 [CAS 71119-22-7, mw. = 231.25]) in 750 mL of dH_2O.

2. Adjust to desired pH using concentrated HCl. Note: This method will produce about 0.24M – 0.88M NaCl in the concentrated stock solution.

3. Fill to final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at 4°C.

Note: Alternatively, equimolar concentrations of MOPS sodium salt and MOPS free acid (MOPS Free Acid, Ultra Pure, GoldBio Catalog # M-790 [CAS 1132-61-2, mw. = 209.26 g/mol]) can be mixed to attain a pH of ~ 7.2. The pH can be adjusted by increasing the molar ratio of MOPS free acid (more acidic) or MOPS sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M MOPS, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 10.32
Adjust pH with: conc. HCl

pH	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9
mL	73	70.5	68	66	63	59	55	51	47	42	37	32	28	24	20

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

MOPS pKa at 25°C: 7.14
MOPS pH range: 6.5 – 7.9
d(pKa)/dT value: -0.011

0.5M MOPSO Buffer - 1 L

Instructions

1. Dissolve 112.63 g of MOPSO, Free Acid (MOPSO, Free Acid, GoldBio Catalog # M-795 [CAS 68399-77-9, mw. = 225.26]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of MOPSO free acid and MOPSO sodium salt (MOPSO, Sodium Salt, GoldBio Catalog # M-799 [CAS 79803-73-9, mw. = 247.24]) can be mixed to attain a pH of ~ 6.9. The pH can be adjusted by increasing the molar ratio of MOPSO free acid (more acidic) or MOPSO sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 0.5M MOPSO, use the table below to estimate the required volume of base for a given pH:

Starting pH: 3.47
Adjust pH with: 10N NaOH

pH	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9
mL	8	10	12	14	16	19	22	25	28	31	33	35	37	39	41

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

MOPSO pKa at 25°C: 6.87
MOPSO pH range: 6.2 – 7.6
d(pKa)/dT value: -0.015

0.5M MOPSO-Na Buffer - 1 L

Instructions

1. Dissolve 123.62 g of MOPSO sodium salt (MOPSO, Sodium Salt, GoldBio Catalog # M-799 [CAS 79803-73-9, mw. = 247.24]) in 750 mL of dH_2O.

2. Adjust to desired pH using concentrated HCl. Note: This method will produce about 0.1M – 0.46M NaCl in the concentrated stock solution.

3. Fill to final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at 4°C.

Note: Alternatively, equimolar concentrations of MOPSO free acid (MOPSO, Free Acid, GoldBio Catalog # M-795 [CAS 68399-77-9, mw. = 225.26]) and MOPSO sodium salt can be mixed to attain a pH of ~ 6.9. The pH can be adjusted by increasing the molar ratio of MOPSO free acid (more acidic) or MOPSO sodium salt (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 0.5M MOPSO, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 10.35
Adjust pH with: conc. HCl

pH	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6
mL	30.8	29.5	28.5	27.3	26	24.4	22.7	20.8	19	17.1	15.1	13.2	11.5	9.6	8.3

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

MOPSO pKa at 25°C: 6.87
MOPSO pH range: 6.2 – 7.6
d(pKa)/dT value: -0.015

1M PIPES Buffer - 1 L

Instructions

1. Add 302.37 g of PIPES (PIPES, Free Acid, GoldBio Catalog # P-281 [CAS 5625-37-6, mw. = 302.37]) to 600 mL of dH_2O. PIPES free acid will not readily dissolve until the solution is raised above pH 6.5.

2. Adjust to desired pH using 10N NaOH.

3. Fill to final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at 4°C.

To make a 1 L solution of 1M PIPES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 2.87
Adjust pH with: 10N NaOH

pH	6.1	6.2	6.3	6.4	*6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5
mL	78	93	108	110	112	115	119	123	127	132	138	144	148	153	158

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

PIPES pKa at 25°C: 6.76
PIPES pH range: 6.1 – 7.5
d(pKa)/dT value: -0.0085

1M PIPES-Na Buffer - 1 L

Instructions

1. Add 335.34 g of PIPES, Sodium Salt (PIPES, Sodium Salt, GoldBio Catalog # P-280 [CAS 100037-69-2, mw. = 335.34]) to 750 mL of dH_2O. The solution should be at pH ~7.2.

2. Adjust to desired pH using 10N NaOH or concentrated HCl. Using HCl to lower the pH will produce 0.044M – 0.44M NaCl in the concentrated stock solution.

3. Fill to final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at 4°C.

To make a 1 L solution of 1M PIPES, use the table below to estimate the required volume of acid or base for a given pH:

Starting pH: 7.2
Adjust pH with: 10N NaOH or conc. HCl

pH	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.3	7.4	7.5
mL	37.2	35.5	34.0	31.4	29.3	25.4	21.6	17.5	13.3	8.2	3.7	10	15	20
	Concentrated HCl											10N NaOH		
	Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.													

PIPES pKa at 25°C: 6.76
PIPES pH range: 6.1 – 7.5
d(pKa)/dT value: -0.0085

1M TAPS Buffer - 1 L

Instructions

1. Dissolve 243.28 g of TAPS (TAPS, GoldBio Catalog # T-780 [CAS 29915-38-6, mw. = 243.28 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 1M TAPS, use the table below to estimate the required volume of base for a given pH:

Starting pH: 2.81
Adjust pH with: 10N NaOH

pH	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0	9.1
mL	6.5	8	10	13	16	19	23	26	32	36	41	47	52	57	62

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

TAPS pKa at 25°C: 8.40
TAPS pH range: 7.7 – 9.1
d(pKa)/dT value: 0.018

1M TES Buffer - 1 L

Instructions

1. Dissolve 229.25 g of TES (TES, GoldBio Catalog # T-785 [CAS 7365-44-8, mw. = 229.25 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 1M TES, use the table below to estimate the required volume of base for a given pH:

Starting pH: 3.83
Adjust pH with: 10N NaOH

pH	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2
mL	8	10	13	15	18	22	27	31	36	41	47	52	57	62	67

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

TES pKa at 25°C: 7.40
TES pH range: 6.8 – 8.2
d(pKa)/dT value: -0.02

1M Tricine Buffer - 1 L

Instructions

1. Dissolve 179.17 g of Tricine (Tricine, GoldBio Catalog # T-870 [CAS 5704-04-1, mw. = 179.17 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using 10N NaOH.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

To make a 1 L solution of 1M Tricine, use the table below to estimate the required volume of base for a given pH:

Starting pH: 5.38
Adjust pH with: 10N NaOH

pH	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8
mL	9	11	14	17	20	24	28	33	38	43	48	54	59	64	69

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

Tricine pKa at 25°C: 8.05
Tricine pH range: 7.4 – 8.8
d(pKa)/dT value: -0.021

1M Tris Buffer - 1 L

Instructions

1. Dissolve 121.14 g of Tris (Tris Base) (Tris, GoldBio Catalog # T-400 [CAS 77-86-1, mw. = 121.14 g/mol]) in 750 mL of dH_2O.
2. Adjust to desired pH using concentrated HCl.
3. Fill to final volume of 1 L with dH_2O.
4. Filter sterilize (recommended) or autoclave.
5. Store at 4°C.

Note: Alternatively, equimolar concentrations of Tris base and Tris HCl (Tris HCl, GoldBio Catalog # T-095 [CAS 1185-53-1, mw. = 157.60]) can be mixed to attain a pH of ~ 8.1. The pH can be adjusted by increasing the molar ratio of Tris HCl (more acidic) or Tris base (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M Tris, use the table below to estimate the required volume of acid for a given pH:

Starting pH: 10.82
Adjust pH with: conc. HCl

pH	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
mL	77	75	73	72	70	67	64	61	57	53	49	45	41	37	32	28	25	21	18	15	12

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

Tris pKa at 25°C: 8.06
Tris pH range: 7.0 – 9.0
d(pKa)/dT value: -0.028

1M Tris HCl Buffer - 1 L

Instructions

1. Dissolve 157.60 g of Tris HCl (Tris HCl, GoldBio Catalog # T-095 [CAS 1185-53-1, mw. = 157.60 g/mol]) in 750 mL of dH_2O.

2. Adjust to desired pH using 10N NaOH. This method will introduce 0.06M - 0.81M NaCl into the concentrated stock solution.

3. Fill to final volume of 1 L with dH_2O.

4. Filter sterilize (recommended) or autoclave.

5. Store at 4°C.

Note: Alternatively, equimolar concentrations of Tris base (Tris, GoldBio Catalog # T-400 [CAS 77-86-1, mw. = 121.14 g/mol]) and Tris HCl can be mixed to attain a pH of ~ 8.1. The pH can be adjusted by increasing the molar ratio of Tris HCl (more acidic) or Tris base (more basic) and estimated using the Hendersen-Hasselbalch equation.

To make a 1 L solution of 1M Tris HCl, use the table below to estimate the required volume of base for a given pH:

Starting pH: 3.58
Adjust pH with: 10N NaOH

pH	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
mL	6	7	9	11	13	16	19	23	27	31	36	41	46	51	56	61	66	70	75	78	81

Note: This data was collected in GoldBio labs using GoldBio reagents and calculated using 100 ml volumes. All reagent volumes recorded above were adjusted accordingly to create this protocol.

Tris pKa at 25°C: 8.06
Tris pH range: 7.0 – 9.0
d(pKa)/dT value: -0.028

Additional Resources

Biological Buffers Resource Page

- Tools
- Protocols
- Articles
- Preparation Instructions
- Reference Charts

Stock Solution Calculator

This calculator allows you estimate how much volume you'll get from a product pack size or weighed mass of powder. Simply select the product solution you're interested in, enter the mass and change units accordingly to get your volume results. This calculator also includes valuable product information including a link to the product, the stock solution recipe and basic information such as catalog number and molecular weight.

Helpful Articles

1. What is a biological buffer and how to choose the best buffer for your experiment
2. Why is my lysis buffer not working: How to resolve 9 lysis buffer issues that might be holding you back
3. How to prepare your most frequently used buffers
4. How to pronounce biological buffers, by GoldBio

GoldBio Buffers by Category

Table 2. Biological Buffers

Buffer	Catalog Number
ACES	A-010
ADA	A-780
BES	B-780
Bicine	B-785
Bis-Tris	B-020
Boric Acid	B-030
CAPS	C-040
CHES	C-870
HEPES, Free Acid	H-400
HEPES, Sodium Salt	H-401
HEPPSO	H-480
MES, Free Acid	M-095
MES Free Acid Monohydrate, Ultra Pure	M-090
MES, Sodium Salt	M-091
MOPS Free Acid, Ultra Pure	M-790
MOPS, Sodium Salt	M-791
MOPSO, Free Acid	M-795
MOPSO, Sodium Salt	M-799
PBS (Phosphate Buffered Saline) Tablets	P-271
PIPES, Sodium Salt	P-280
TAPS	T-780
TES	T-785
Tricine	T-870
Tris Acetate	T-090
Tris HCl	T-095
Tris (Tris Base)	T-400

Table 3. Chromatography Buffers

Buffer	Catalog Number
5X Phosphate Buffer + 3M Imidazole Solution	I-905
5X Phosphate Buffer, pH 8	P-500
His-Tag Buffer Set	I-906
Imidazole - 3M Recrystallized Solution, pH 6.0	I-901
Imidazole, Recrystallized	I-902

Table 4. Cell Culture Buffers

Buffer	Catalog Number
ACES	A-010
BES	B-780
Bicine	B-785
HEPES, Free Acid	H-400
HEPES, Sodium Salt	H-401
MES Free Acid Monohydrate, Ultra Pure	M-090
MOPS Free Acid, Ultra Pure	M-790
MOPSO, Free Acid	M-795
PBS (Phosphate Buffered Saline) Tablets	P-271
PIPES, Sodium Salt	P-280
TAPS	T-780
Tricine	T-870

Table 5. Electrophoresis Buffers

Buffer	Catalog Number
Bis-Tris	B-020
Boric Acid	B-030
Glycine, sodium salt	G-450
Tricine	T-870
Tris Acetate	T-090
Tris HCl	T-095
Tris (Tris Base)	T-400

Table 6. Lysis Buffers

Buffer	Catalog Number
Bacterial Cell Lysis Buffer	GB-176, GB-177
Mammalian Cell Lysis Buffer	GB-180
Tissue Cell Lysis Buffer	GB-181
Yeast Lysis Buffer Kit plus Zymolyase	GB-178
Yeast Spheroplast Lysis Buffer	GB-179

Biological Buffer Selection Guide

Buffer	Catalog #	pH Range	pKa (25°C)	Applications	Metal Binding	Comments
• ACES	A-010	6.1 – 6.8	6.8	Cell culture media, agarose and polyacrylamide electrophoresis, isoelectric focusing of proteins, x-ray crystallography, and yeast and bacterial cell studies.	Binds Cu^{2+} and Mg^{2+}.	Significant absorption of UV light at 230 nm. Inhibits GABA receptor binding.
• ADA	A-780	6.0 – 7.2	6.6	Protein crystallization, electrophoresis, differential scanning calorimetry and metal decontamination in soil.	Strong binding to Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+}.	Absorbs UV light between 0.1 and 260 nm. Not suitable for BCA assay.
• BES	B-780	6.4 – 7.8	7.1	Transfection, cell culture and protein quantification. Chromatography (gel filtration and cation exchange). Bacterial endotoxin studies.	Binds Cu^{2+} and Co^{2+} weakly.	Suitable for BCA assay. Binds DNA and interferes with restriction enzymes.
• Bicine	B-785	7.6 – 9.0	8.3	Tissue culture, phosphorylation, protein crystallization, enzymatic reactions, a multiphasic buffer for SDS-PAGE. Cation exchange chromatography and NMR spectroscopy.	Strong binding to Mg^{2+}, Ca^{2+}, Co^{2+}, Fe^{3+} and Cu^{2+} Weak binding to Mn^{2+}.	Zwitterionic buffer. Not suitable for BCA assay.
• Bis-Tris	B-020	5.8 – 7.2	6.5	Electrophoresis (sample, gel and running buffer), anion exchange chromatography, protein purification, NMR spectroscopy and x-ray crystallography.	Binds Cu^{2+} and Pb^{2+}. Weak binding with other metals.	Zwitterionic buffer. Substitute for cacodylate buffer. Interacts with human liver FAB and affects protein dynamics. Not suitable for BCA assay.
• Boric Acid	B-030	8.2 – 10.2	9.2	Capillary and gel electrophoresis (protein, nucleic acid and secondary metabolite separation). Plant micronutrient.		Binds carbohydrates and pyridine nucleotides, such as NAD^{+}.

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Biological Buffer Selection Guide (part 2)

Buffer	Catalog #	pH Range	pKa (25°C)	Applications	Metal Binding	Comments
CAPS	C-040	9.7 - 11.1	10.4	SDS-PAGE, protein sequencing and quantification and enzymatic assays. Cation exchange chromatography and protein crystallization.	Negligible metal ion binding	Zwitterionic buffer. Suitable for BCA assay.
CHES	C-870	8.6 - 10.0	9.5	Electrophoresis and enzymatic assays. Electron paramagnetic resonance (EPR) spectroscopy, kinetic measurements, yeast dextrose medium.	Negligible metal ion binding	Zwitterionic buffer. High affinity for iodoacetate binding site of liver alcohol dehydrogenase.
HEPES Free Acid HEPES Sodium Salt	H-400 H-401	6.8 - 8.2	7.5	Cell culture, electroporation, qPCR, restriction enzymes, atomic force microscopy and electrophoresis. Protein quantification. Cation exchange chromatography.	No binding	Zwitterionic buffer. May form radicals and not suitable for redox reactions. Binds DNA and affects restriction enzyme function.
HEPPSO	H-480	7.1 - 8.5	7.8	Protein quantification. Toxicology studies. Isoelectric focusing and enzymatic studies.	Weak binding with Cu^{2+}	May form radicals and not suitable for redox reactions. Suitable for BCA assay.
MES Free Acid MES Free Acid Monohydrate, Ultra Pure MES Sodium Salt	M-095 M-090 M-091	5.5 - 6.7	6.1	Cell and plant culture media, protein electrophoresis, chromatography, electrochromatography. Toxicology studies in yeast (metal toxicity and removal). Fluorescence microscopy.	Negligible binding	Zwitterionic buffer. Substitute for cacodylate, citrate and malate buffers. Inhibits connexin channels. Binds human liver FAB protein.
MOPS Free Acid MOPS Sodium Salt	M-790 M-791	6.5 - 7.9	7.1	Cell culture, nucleic acid/ protein electrophoresis, and protein purification and quantification. Toxicology studies in yeast (metal toxicity).	Negligible binding	Zwitterionic buffer. Filter sterilization required. Suitable for BCA assay.

Biological Buffer Selection Guide (part 3)

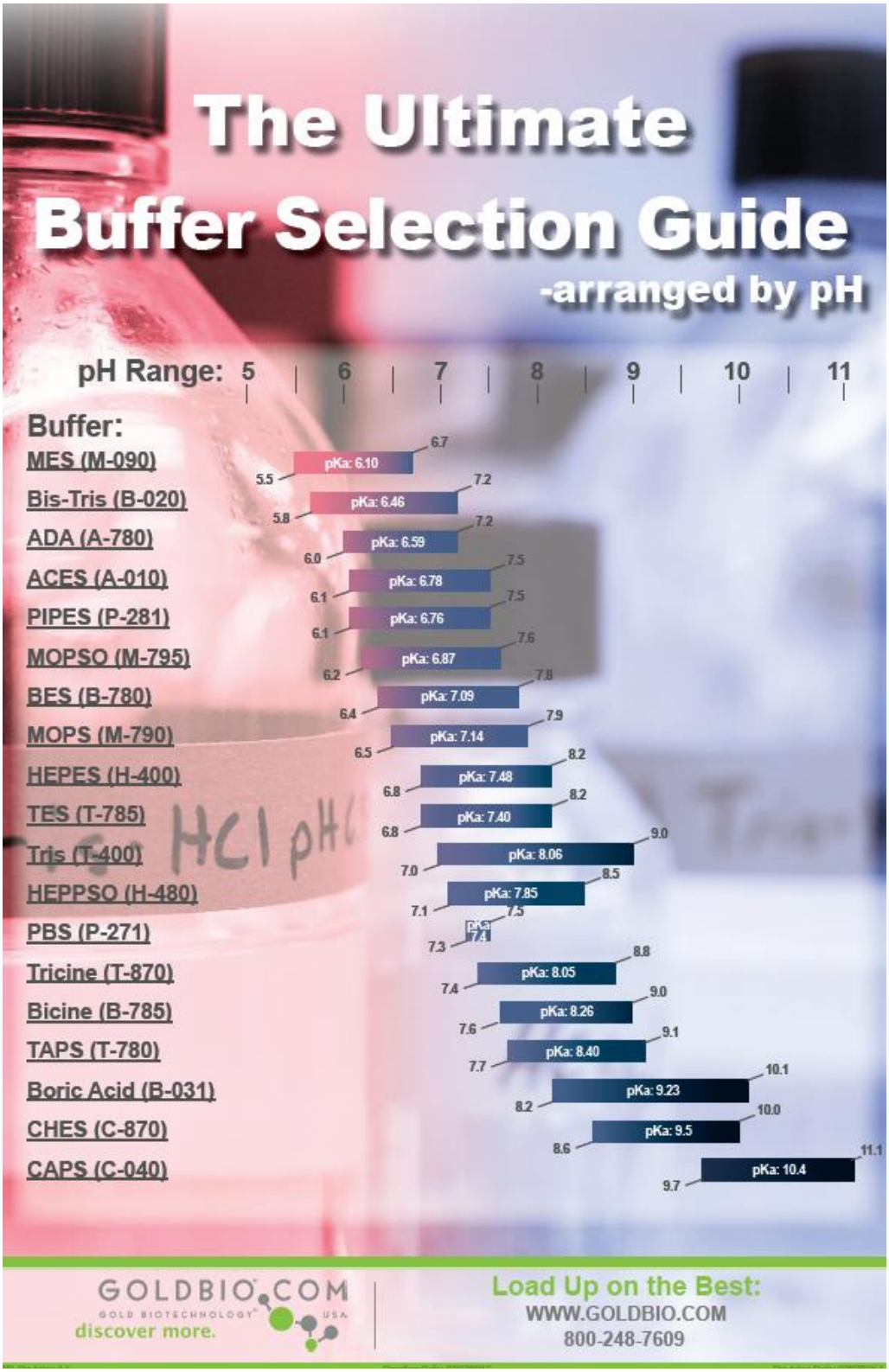
Buffer	Catalog #	pH Range	pKa (25°C)	Applications	Metal Binding	Comments
MOPSO Free Acid MOPSO Sodium Salt	M-795 M-799	6.2 - 7.6	6.9	Cell culture media, capillary electrophoresis and quantification. Fluorescence spectroscopy, spectrophotometry and isothermal titration calorimetry. Electrochromatography.	Binds Fe^{3+}.	Zwitterionic buffer. PTFE-coated or plastic coated utensils recommended for its use.
PIPES Free Acid PIPES Sodium Salt	P-281 P-280	6.1 - 7.5	6.8	Cell culture, protein crystallization, electrophoresis, isoelectric focusing. Chromatography and protein quantification. Electron microscopy.	Negligible binding	Zwitterionic buffer. May form radicals and not suitable for redox reactions. Suitable for BCA assay. Substitute for cacodylate.
TES	T-785	6.8 - 8.2	7.4	DNA precipitation and extraction. Gel filtration and chromatography. Enzyme assays. Protein quantification.	Binds strongly to Cr^{3+} and Fe^{3+}, and binds weakly to Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}	Zwitterionic buffer. Suitable for BCA assay.
Tricine	T-870	7.4 - 8.8	8.1	SDS-PAGE, capillary zone electrophoresis, HPLC and ion exchange chromatography, firefily luciferase assays. Lowry protein assay upon addition of	Binds Cu^{2+} strongly, Ca^{2+} and Mg^{2+} moderately and weakly to Mn^{2+}	Substitute for barbital. Photooxidized by flavins and flavoproteins.
Tris (Tris Base) Tris-HCl	T-400 T-095	7.0 - 9.0	8.1	Electrophoresis, SDS-PAGE Laemmli buffer and other running/loading buffers with glycine, and SDS. Anion exchange chromatography.	Strong binding to Fe^{3+}, Cr^{3+}, Ni^{2+} Co^{2+}, Cu^{2+}	Reacts with DEPC, aldehydes, common metals, various enzymes and DNA. Not suitable for BCA assay. Not suitable for most cell culture work.
TAPS	T-780	7.7 - 9.1	8.4	DNA analysis, protein purification (ion exchange chromatography, planar chromatography), enzymatic assay (aminopeptidase activity), cell culture media (dinoflagellates).	Binds strongly to Cr^{3+}, Fe^{3+} and Cu^{2+}	Inhibits connexin channels.

Guide: Commonly used Buffers

Buffer	pH Range	Applications	Comments
PBS (Phosphate Bufferd Saline)	7.3 - 7.5	Cell culture, immunoassays, sample dilution and protein purification.	Isotonic and nontoxic buffer. Mimics pH, osmolarity and ion concentration of the human body. pKa 7.4. GoldBio Catalog # P-271.
Cacodylate	5.0 - 7.4	Electron microscopy and histology.	Substitute for Sorensen's buffer. Toxic. At low pH, oxidizes thiol groups and can inactivate enzymes that require reduced thiols. pKa 6.27.
Stripping buffer		Western blotting.	Components: Tris-HCl 1M, 20% SDS and β-mercaptoethanol. Prepare fresh prior to use. If antibody recovery from the membrane is desired, avoid SDS.
Transfer buffer		Western blotting.	Components: Tris-base 25mM, glycine 192mM, and 20% methanol.
Running buffer	8.3	Western blotting.	Components: Tris-base 25mM, glycine 192mM, and 0.1% SDS. Can be stored at room temperature. No pH adjustment is required.
Krebs-Henseleit bicarbonate buffer	7.4	Perfusion and isolation of muscle tissue.	Components: NaCl 118mM, KCl 4.7mM, $MgSO_4$ 1.2mM, $CaCl_2$ 2.5mM and $NaHCO_3$ 25mM. Equilibrate with 95% O_2 and 5% CO_2. Add $CaCl_2$ after all other compounds are dissolved. Add glucose for cell maintenance. Use immediately after preparation.
TE (Tris-EDTA buffer)	7.9 - 8.1	Storage of nucleic acids.	Components: Tris-Cl 10mM pH 7.4-8.0, EDTA 1mM pH 8.0. EDTA might affect downstream applications. Can be stored at room temperature for up to 6 months.

Guide: Commonly used Buffers (part 2)

Buffer	pH Range	Applications	Comments
TAE (Tris-acetate-EDTA buffer)	8.0 - 8.3	Agarose electrophoresis for the separation of nucleic acids (DNA and RNA).	Components: Tris base 40mM, acetic acid 20mM and 1mM EDTA. TAE has a lower buffering capacity than TBE. Can be stored at room temperature. Stock solution protocol.
TTE (Tris-TAPS-EDTA buffer)	8.3	Electrophoresis.	Components: 500mM Tris base, 500mM TAPS and 10mM EDTA. Can be stored at room temperature for up to 4 weeks or at 4°C for long term storage. Stock solution protocol.
TBE (Tris-borate-EDTA buffer)	8.3	Polyacrylamide gel electrophoresis for the separation of nucleic acids (DNA and RNA). blotting.	Components: Tris base 89mM, boric acid 89mM and EDTA 2mM. Used with denaturing and non-denaturing gels. Borate strongly inhibits enzymatic activity. Stock solution protocol.
TBST (Tris buffered saline with Tween® 20)	7.4 - 7.6	Western blotting and ELISA.	Components: Tris HCl 20mM pH 7.5, NaCl 150mM and 0.05-0.1% Tween® 20.
Tris-Glycine	8.3	Western blotting, SDS-PAGE, ion-exchange chromatography.	Components: Tris-base 25mM, glycine 250mM and 0.1% SDS.
Imidazole-HCl	6.2 - 7.8	Affinity chromatography and SDS-PAGE.	Binds various metals. Unstable.



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