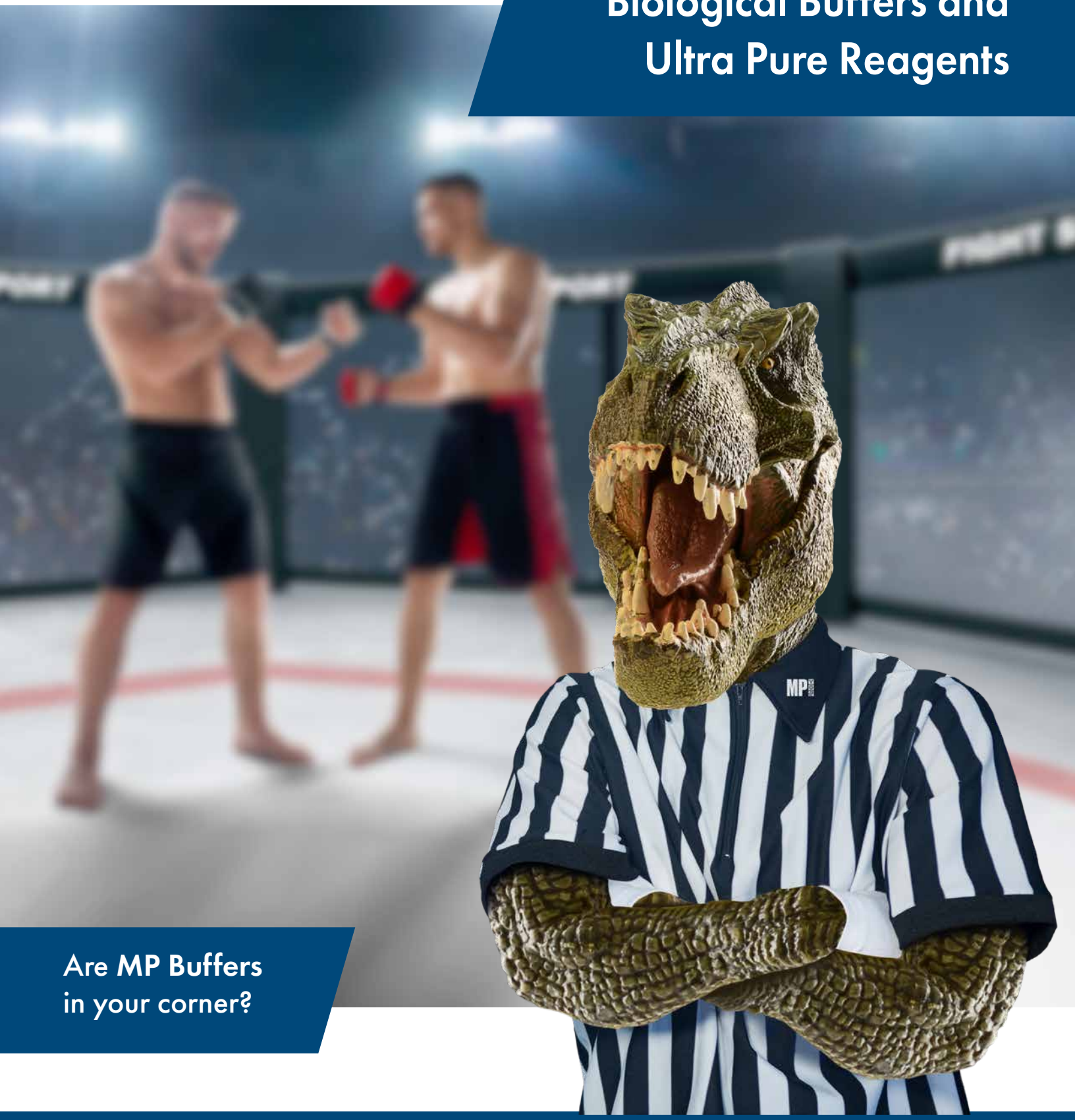
Biological Buffers and Ultra Pure Reagents



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Theoretical Considerations

Since buffers are essential for controlling the pH in many biological and biochemical reactions, it is important to have a basic understanding of how buffers control the hydrogen ion concentration. Although a lengthy, detailed discussion is impractical, some explanation of the buffering phenomena is important.

Let us begin with a discussion of the equilibrium constant (K) for weak acids and bases. Acids and bases which do not completely dissociate in solution, but instead exist as an equilibrium mixture of undissociated and dissociated species, are termed weak acids and bases. The most common example of a weak acid is acetic acid. In solution, acetic acid exists as an equilibrium mixture of acetate ions, hydrogen ions, and undissociated acetic acid. The equilibrium between these species may be expressed as follows:

$$HAc \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H^+ + Ac^-$$

where k_1 is the dissociation rate constant of acetic acid to acetate and hydrogen ions and k_2 is the association rate constant of the ion species to form acetic acid. The rate of dissociation of acetic acid, -d(HAc)/dt, may be expressed by the following equation:

$$-\frac{d(HAc)}{dt} = k_1 (HAc)$$

which shows the rate of dissociation to be dependent upon the rate constant of dissociation (k_1) and the concentration of acetic acid (HAc).

Similarly, the rate association, d(HAc)/dt, which is dependent upon the rate constant of association (k_2) and the concentration of acetate and hydrogen ions, may be shown as:

$$\frac{d(HAc)}{dt} = k_2 (H^+)(Ac^-)$$

Since, under equilibrium conditions, the rates of dissociation and association must be equal, they may be expressed as:

$$k_1 (HAc) = k_2 (H^+)(Ac^-)$$

Or

$$\frac{k_1}{k_2} = \frac{(H^+)(Ac^-)}{(HAc)}$$

If we now let $k_1/k_2 = K_a$, the equilibrium constant, the equilibrium expression becomes:

$$K_a = \frac{(H^+)(Ac^-)}{(HAc)}$$

which may be rearranged to express the hydrogen ion concentration in terms of the equilibrium constant and the concentrations of undissociated acetic acid and acetate ions as follows:

$$(H^+) = K_a \frac{(HAc)}{(Ac^-)}$$

Since pH is defined as -log (H^+), if the equilibrium expression is converted to -log:

$$-\log (H^+) = -\log K_a - \log \frac{(HAc)}{(Ac^-)}$$

And by substituting pH and pK_a :

$$pH = pK_a - \log \frac{(HAc)}{(Ac^-)}$$

Or

$$pH = pK_a + \log \frac{(Ac^-)}{(HAc)}$$

When the concentration of acetate ions equals the concentration of acetic acid, log (Ac^-)/(HAc) becomes zero, and the pH equals pK_a. As a result, the pK_a of a weak acid or base generally indicates the pH of the center of the buffering region.

pK_a values are generally determined by titration. The free acid of the material to be measured is carefully titrated with a suitable base, and using a calibrated automatic recording titrator, the titration curve is recorded. A general titration curve for a typical monobasic weak acid is shown in Figure 1. The point of inflection indicates the pK_a value.

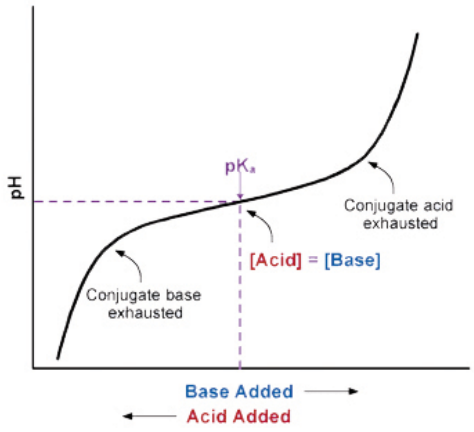

Figure 1.
Typical Titration Curve of a weak acid

Using acetic acid as an example, it has now been demonstrated that pH = pK_a when the concentrations of acetic acid and acetate ions are equal. This buffering action helps explain how the hydrogen ion concentration (H^+) remains relatively unaffected by external influences. Let's look at a hypothetical buffer system, HA (pK_a = 7.000) and (A^-). If we consider a non-buffered system to which a strong acid is added, we can observe a significant change in pH. For example, if 100 mL of 1.000 x 10^{-2} M HCl are added to 1.000 liter of 1.000 M NaCl at pH 7.000, the hydrogen ion concentration $(H^+)_f$ of the final 1.100 liters of solution may be calculated by:

$$(H^+)_f \times Vol_f = (H^+)_i \times Vol_i$$

$$(H^+)_f \times 1.100 = 1.000 \times 10^{-2} \times 0.100$$

$$(H^+)_f = 9.09 \times 10^{-4}$$

$$-\log (H^+)_f = -\log (9.09 \times 10^{-4})$$

$$pH = 3.04$$

Thus, it can be observed that the addition of 1.0 x 10^{-3} moles of hydrogen ion to the unbuffered system resulted in a change in pH from 7.000 to 3.04.

Now, using the hypothetical buffer system, a 1.000 M solution of HA at pH 7.000 can be shown initially as:

$$(HA) = (A) = 0.500\ M$$

$$pH = pK + \log \frac{(A)}{(HA)}$$

$$pH = 7.000 + \log \frac{0.500}{0.500}$$

$$pH = 7.000$$

If we add to this system 100 mL of 1.000 x 10^{-2} M HCl, 1.000 x 10^{-3} moles of A must be converted to 1.000 x 10^{-3} moles of HA. The resulting equation thus becomes:

$$pH = 7.000 + \log \frac{0.499/1.100}{0.501/1.100}$$

$$pH = 7.000 - 0.002$$

$$pH = 6.998$$

So it can be seen that in the buffered system the pH has changed by only 0.002 pH units, compared to a change of almost 4 pH units in the unbuffered system.

In summary, the principles involved in hydrogen ion buffer systems have been very basically illustrated. Beginning with an understanding of equilibrium, pH and pK_a, we have attempted to demonstrate how buffering capacity is determined and how a buffered system may effectively resist changes in pH.

Practical Considerations

The need for buffers in biological and biochemical research is universal. However, in the past, very few buffers in the important pH range of 6 to 8 were available. Those that were available were inappropriate for biological research and had serious disadvantages, such as toxicity or undesired reactivity. Phosphate buffers, for example, exhibit poor buffering capacity above pH 7.5, and they often inhibit reactions and precipitate polyvalent cations. Below pH 7.5, buffers such as TRIS can be toxic and show poor buffering capacity. Similarly, glycylglycine is useful above pH 8, but is of no value below pH 7.5.

Several important criteria must be met in order for a buffer to be useful in biological systems:

1. **The buffers must be enzymatically and hydrolytically stable.**
2. **The pK_a of the buffer should be between 6 and 8 for most biological reactions.**
3. **The pH of the buffer solution should be minimally affected by concentration, temperature, ionic composition, or salt effects of the medium.**
4. **The buffer should be soluble in water and relatively insoluble in other solvents.**
5. **Cationic complexes should be soluble.**
6. **The buffer should exhibit no absorption of light in either the visible or UV regions.**

Some years ago, Good[1] described a series of zwitterionic buffers possessing these characteristics. These so-called "Good's Buffers" are now widely used in cell culture, electrophoresis, biological systems and biochemical reactions. Over the years, several new zwitterionic buffers have been added to the original list of Good's buffers, and a list of these is shown in Table 1.

pk_a	Buffer	Cat. No.	pH Range	MW	Water Solubility (0°C, gm/100 mL)
6.15	MES	195309	5.8 - 6.5	195.2	12.7
6.50	BIS-TRIS	101038	5.8 - 7.2	209.2	20.9
6.62	ADA	150223	6.2 - 7.2	190.1	1.7
6.80	BIS-TRIS Propane	152447	6.3 - 9.5	282.3	42.8
6.76	PIPES	190257	6.1 - 7.5	302.4	slightly
6.80	MOPSO	151707	6.2 - 7.4	225.3	22.5
6.88	ACES	100011	6.4 - 7.4	182.2	6.6
7.15	BES	100927	6.6 - 7.6	213.2	68.2
7.20	MOPS	102370	6.5 - 7.9	209.3	6.5
7.50	TES	103008	7.0 - 8.0	229.2	59.6
7.55	HEPES	101926	7.0 - 8.0	238.3	53.6
7.60	TAPSO	152459	7.0 - 8.2	259.3	13.0
7.80	HEPPSO	151236	7.1 - 8.5	268.3	26.8
8.00	HEPPS	101927	7.6 - 8.6	252.3	39.9
8.10	TRIS	152176	7.0 - 9.0	121.1	50.0
8.15	TRICINE	103112	7.6 - 8.8	179.2	14.3
8.35	BICINE	101005	7.8 - 8.8	163.2	18.0
8.40	TAPS	103007	7.7 - 9.1	243.3	5.0
9.55	CHES	101434	9.0 - 10.1	207.3	23.6
9.60	CAPSO	152448	8.9 -10.3	237.3	11.1
10.40	CAPS	101435	9.7 -11.1	221.3	10.4

Table 1.
Biological and Biochemical Buffers

Zwitterionic buffers are typically supplied in the free acid form, although several are available as sodium salts, to aid in their solubility. As a general rule, a buffer is chosen so that the pK_a is slightly below the desired pH. By then adjusting with a suitable base, the buffer is brought to the desired pH.

Tissue Culture Applications

Several of the Good's buffers, most notably HEPES, TRICINE and TES, have been shown to be very effective in cell culture. Ceccarini and Eagle[2] have studied the optimum pH for growth of a number of normal, virus-transformed, and cancer cells, using various zwitterionic buffers to stabilize pH.

A study by Eagle[3] has shown that eight of the Good's buffers are non-toxic. These buffers include BIS-TRIS, PIPES, BES, TES, HEPES, HEPPS, TRICINE and Bicine. A table of suggested buffer combinations for use in the presence of bicarbonate is also presented in Eagle's study.

In a study by Shipman[4], HEPES was found to give higher maximum cell densities and viabilities in cultures, such as human embryonic lung, chick embryo fibroblast and guinea pig spleen cells. In viral studies, Shipman also observed that HEPES-buffered saline did not affect Rubella virus titration or hemagglutination assays for Polyoma or Sendai viruses. Phosphate-buffered saline had been reported to affect these determinations.

Description	CAS #	Formula	MW	Size	Cat. No.
ACES [N-(2-Acetamido)-2-aminoethanesulfonic acid]. pK_a = 6.88. Useful pH range 6.4–7.4. One of Good's zwitterionic buffers used for both agarose and PAGE electrophoresis applications, as well as in isoelectric focusing of proteins.	[7365-82-4]	$C_4H_{10}N_2O_4S$	182.2	25 g 100 g 250 g	100011
ACES, ULTRA PURE [N-(2-Acetamido)-2-aminoethanesulfonic acid]. Purity: >99%. pK_a = 6.88. Useful pH range 6.4–7.4. Useful for isoelectric focusing of proteins and as a buffering component in cell culture media.	[7365-82-4]	$C_4H_{10}N_2O_4S$	182.2	5 g 25 g 250 g	193973
ADA [N-(2-Acetamido)-2-iminodiacetic acid]. pK_a = 6.62. Useful pH range 6.2–7.2. A zwitterionic buffer useful in cell culture applications due to its physiological pH range. Also used as a complexing agent to remove contaminant metals from soil.	[26239-55-4]	$C_6H_{10}N_2O_5$	190.2	25 g 250 g	150223
BES [N,N-bis(2-Hydroxyethyl)-2-aminoethanesulfonic acid]. Free Acid. pK_a = 7.15. Useful pH range 6.6–7.6. BES buffer has been used in calcium phosphate-mediated transfection of eukaryotic cells with plasmid DNA.	[10191-18-1]	$C_6H_{15}NO_5S$	213.3	5 g 25 g 100 g 250 g 1 kg	100927
BES, SODIUM SALT [N,N-bis(2-Hydroxyethyl)-2-aminoethanesulfonic acid]. Sodium salt. pK_a = 7.15 (free acid). Useful pH range 6.4–7.8. A readily soluble form of BES buffer.	[66992-27-6]	$C_6H_{15}NO_5SNa$	235.2	25 g 100 g 500 g	152446
BICINE [N,N-bis(2-Hydroxyethyl)glycine]. pK_a = 8.35. Useful pH range 7.8–8.8. BICINE is used in protein crystallization, studying enzyme reactions and electrophoresis.	[150-25-4]	$C_6H_{13}NO_4$	163.2	25 g 100 g 500 g 1 kg	101005

Practical Considerations

Description	CAS #	Formula	MW	Size	Cat. No.
BIS-TRIS [2,2-bis(Hydroxymethyl)-2,2',2''-nitrilotriethanol]. pK_a = 6.50. Useful pH range 5.8–7.2. A zwitterionic buffer used to calibrate glass electrodes and for nucleic acid and protein crystallizations.	[6976-37-0]	$C_8H_{19}NO_5$	209.2	25 g 100 g 500 g 1 kg	101038
BIS-TRIS PROPANE [1,3-bis{tris(Hydroxymethyl)methylamino}-propane]. pK_a 1 = 6.8 and pK_a 2 = 9.0. Useful pH range 6.3–9.5. A buffer with a wide buffering range due to its two pKa values. Has been used to enhance stability of restriction enzymes at low pH and for diagnostic assay manufacturing.	[64431-96-5]	$C_{11}H_{26}N_2O_6$	282.3	10 g 25 g 100 g 250 g	152447
CAPS [3-(Cyclohexylamino)propanesulfonic acid]. pK_a = 10.4. Useful pH range 9.7–11.1. A zwitterionic buffer used for protein sequencing and identification, Western blotting and immunoblotting.	[1135-40-6]	$C_9H_{19}NO_3S$	221.3	25 g 100 g 250 g 1 kg	101435
CAPSO [3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid]. pK_a = 9.60. Useful pH range 8.9–10.3. Used in protein sequencing, Western and immunoblotting procedures. Especially effective for transferring proteins with pI > 8.5 to PVDF and nitrocellulose membranes.	[73463-39-5]	$C_9H_{19}NO_4S$	237.3	25 g 100 g 500 g	152448
CAPSO SODIUM SALT [3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid sodium salt]. Sodium Salt. pK_a = 9.60. Useful pH range 8.9–10.3. CAPSO is the buffer of choice for Western and immunoblotting of strongly basic proteins . Especially effective for transferring proteins with pI > 8.5 to PVDF and nitrocellulose membranes. The sodium salt form has slightly better solubility than CAPSO free acid.	[102601-34-3]	$C_9H_{19}NO_4SNa$	259.3	25 g 100 g	152449
CHES [2-(Cyclohexylamino)ethanesulfonic acid]. pK_a = 9.55. Useful pH range 9.0–10.1. Typically used to study enzymatic processes above physiological pH.	[103-47-9]	$C_8H_{17}NO_3S$	207.3	25 g 100 g	101434
HEPES (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid). pK_a = 7.55. Useful pH range 7.0–8.0. A zwitterionic Good's buffer widely used in cell culture media and as an ampholytic separator to create a pH gradient in isoeletric focusing.	[7365-45-9]	$C_8H_{18}N_2O_4S$	238.3	25 g 100 g 250 g 1 kg	101926
HEPES HEMISODIUM SALT (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid). Hemisodium salt. pK_a = 7.5. Useful pH range 6.8–7.2. Zwitterionic buffer widely used to maintain physiological pH, with slightly better solubility than HEPES free acid.	[103404-87-1]	$C_8H_{17}N_2O_4 \bullet 1/2Na$	249.3	25 g 100 g	152451

Description	CAS #	Formula	MW	Size	Cat. No.
HEPES SODIUM SALT (N-2-Hydroxyethylpiperazine-N'-3-ethanesulfonic acid). Sodium salt. pK_a = 7.5. Useful pH range 6.8–7.2. Zwitterionic buffer widely used to maintain physiological pH, with slightly better solubility than HEPES free acid.	[75277-39-3]	$C_8H_{17}N_2O_4Na$	260.3	25 g 100 g 250 g 1 kg	105593
HEPPS (N-2-Hydroxyethylpiperazine-N'-3-propanesulfonic acid). pK_a = 8.00. Useful pH range 7.6–8.6. This is the propane analog of HEPES and has many similar properties. Suitable for use in phosphorylation reactions when metal binding may occur. In mice it has been shown to break-up amyloid beta plaques associated with Alzheimer's Disease.	[16052-06-5]	$C_9H_{20}N_2O_4S$	252.3	25 g 100 g 250 g 1 kg	101927
HEPPSO [4-(2-Hydroxyethyl)piperazine-1-(2-hydroxypropanesulfonic acid)]. pK_a = 7.80. Useful pH range 7.1–8.5. Zwitterionic buffer commonly used as an ampholytic separator to create a pH gradient in isoelectric focusing.	[68399-78-0]	$C_9H_{20}N_2O_5S$	268.3	10 g 25 g 100 g	151236
MES [2-(N-Morpholino)ethanesulfonic acid]. Monohydrate. pK_a = 6.15. Useful pH range 5.8–6.5. A zwitterionic buffer used in SDS-PAGE applications, preparation of culture media, and fluorescence microscopy. One of the first Good's buffers used for protein purification.	[4432-31-9]	$C_6H_{13}NO_4S \bullet H_2O$	213.2	25 g 100 g 250 g 1 kg	195309
MES SODIUM SALT [2-(N-Morpholino)ethanesulfonic acid]. Sodium salt. pK_a = 6.15. Useful pH range 5.8–6.5. A zwitterionic buffer used in SDS-PAGE applications, preparation of culture media, and fluorescence microscopy. One of the first Good's buffers used for protein purification.	[71119-23-8]	$C_6H_{12}NO_4SNa$	217.2	10 g 100 g	152454
MOPS [3-(N-Morpholino)propanesulfonic acid]. Free Acid. pK_a = 7.20. Useful pH range 6.5–7.9. Widely used zwitterionic buffer due to its inert properties. It does not interact with any metal ions in solution. Used in mammalian cell culture and denaturing gel electrophoresis of RNA. Interacts with BSA and stabilizes it.	[1132-61-2]	$C_7H_{15}NO_4S$	209.3	25 g 100 g 250 g 1 kg	102370
MOPS SODIUM SALT [3-(N-Morpholino)propanesulfonic acid]. Sodium Salt. pK_a = 7.20. Useful pH range 6.5–7.9. Widely used zwitterionic buffer in cell culture. MOPS can modify lipid interactions and influence the thickness and barrier properties of membranes. Interacts with BSA and stabilizes it.	[71119-22-7]	$C_7H_{14}NO_4SNa$	231.2	25 g 100 g 250 g 1 kg	190670

Description	CAS #	Formula	MW	Size	Cat. No.
MOPSO [3-(N-Morpholino)- 2-hydroxypropane sulfonic acid]. Free Acid. pK_a = 6.80. Useful pH range 6.2–7.4. A zwitterionic buffer commonly used for cell culture media, as a running buffer in electrophoresis, and for protein purification. MOPSO has low ionic mobility, does not form complexes with most metals, and interacts with the peptide backbone of bovine serum albumin (BSA) to stabilize BSA against thermal denaturation.	[68399-77-9]	$C_7H_{15}NO_5S$	225.3	25 g 100 g 1 kg	151707
MOPSO SODIUM SALT [3-(N-Morpholino)- 2-hydroxypropane sulfonic acid]. Sodium Salt. pK_a = 6.90. Useful pH range 6.2–7.6. A zwitterionic buffer commonly used for cell culture media, as a running buffer in electrophoresis, and for protein purification. Although MOPSO does not form complexes with most metals, it may have a strong interaction with iron in solution.	[79803-73-9]	$C_7H_{14}NO_5SNa$	247.2	25 g 100 g	152455
PIPES [Piperazine-N,N'-bis(2-ethanesulfonic acid]. Free Acid. pK_a = 6.76. Useful pH range 6.1–7.5. A zwitterionic buffer used in cell culture and protein purification. PIPES can minimize lipid loss when buffering glutaraldehyde histology in plant and animal tissues.	[5625-37-6]	$C_8H_{18}N_2O_6S_2$	302.4	25 g 100 g 500 g 1 kg	190257
PIPES DISODIUM SALT [Piperazine-N,N'-bis(2-ethanesulfonic acid]. Disodium Salt. pK_a = 6.76. Useful pH range 6.1–7.5. A zwitterionic buffer used in cell culture and protein purification. PIPES can minimize lipid loss when buffering glutaraldehyde histology in plant and animal tissues.	[76836-02-7]	$C_8H_{16}N_2O_6S_2Na_2$	346.3	25 g 100 g 500 g 1 kg	102660
PIPES SESQUISODIUM SALT [Piperazine-N,N'-bis(2-ethanesulfonic acid]. Sesquisodium Salt. pK_a = 6.76. Useful pH range 6.1–7.5. A zwitterionic buffer commonly used in cell culture media, in protein crystallization, as a running buffer in gel electrophoresis, and as an eluent in isoelectric focusing and chromatography. Contains 1.5 moles sodium per mole PIPES.	[100037-69-2]	$C_8H_{16.5}N_2O_6S_2Na_{1.5}$	335.3	10 g 100 g	152450
TAPS [N-tris(Hydroxymethyl)methyl-3-aminopropanesulfonic acid]. Free Acid. pK_a = 8.40. Useful pH range 7.7–9.1. A zwitterionic buffer used in DNA electrophoresis and in planar chromatography to separate dyes. TAPS inhibits connexin channels and is the preferred culture media buffer used for dinoflagellate experiments.	[29915-38-6]	$C_7H_{17}NO_6S$	243.3	25 g 100 g	103007

Description	CAS #	Formula	MW	Size	Cat. No.
TAPSO [3-(N-tris(Hydroxymethyl)methylamino-2-hydroxypropanesulfonic acid]. Free Acid. pK_a = 7.60. Useful pH range 7.0–8.2. The hydroxy analog of TAPS. TAPSO is used in cell culture media formulations.	[68399-81-5]	$C_7H_{17}NO_7S$	259.3	25 g 100 g	152459
TES [N-tris(Hydroxymethyl)methyl-2-aminoethanesulfonic acid]. Free Acid. pK_a = 7.50. Useful pH range 7.0–8.0. A zwitterionic buffer used in cell culture formulations.	[7365-44-8]	$C_6H_{15}NO_6S$	229.2	25 g 100 g 500 g 1 kg	103008
TES SODIUM SALT [N-tris(Hydroxymethyl)methyl-2-aminoethanesulfonic acid]. Sodium Salt. pK_a = 7.50. Useful pH range 7.0–8.0. A zwitterionic buffer used in cell culture formulations.	[70331-82-7]	$C_6H_{14}NO_6SNa$	251.2	25 g 100 g 250 g	152461
TRICINE [N-tris(Hydroxymethyl)methylglycine]. pK_a = 8.15. Useful pH range 7.6–8.8. A zwitterionic buffer used in SDS-PAGE procedures to separate low molecular weight peptides.	[5704-04-1]	$C_6H_{13}NO_5$	179.2	25 g 100 g 250 g 1 kg	103112
TRIS [Tris-(hydroxymethyl)aminomethane; Tromethamine; Trometamol]. Purity: 99.0–99.5%. pK_a = 8.1. Useful pH range 7.0–9.0. Widely used buffer component for buffer solutions and protein purification. This grade of TRIS is excellent where purity and value are both important. It is superior to technical grade and less expensive than Ultra Pure material.	[77-86-1]	$C_4H_{11}NO_3$	121.1	100 g 500 g 1 kg 5 kg	152176
TRIS USP [Tris-(hydroxymethyl)aminomethane; Tromethamine; Trometamol]. USP Grade. Purity: 99.95% minimum. pK_a = 8.1. Useful pH range 7.0–9.0. Excellent biochemical and biological buffer where certified high purity is required.	[77-86-1]	$C_4H_{11}NO_3$	121.1	100 g 500 g 1 kg	195605
TRIS ULTRA PURE [Tris-(hydroxymethyl)aminomethane; Tromethamine; Trometamol]. Ultra Pure Grade. Purity: 99.95% minimum. pK_a = 8.1. Useful pH range 7.0–9.0. Excellent biochemical and biological buffer for all applications where high purity is required.	[77-86-1]	$C_4H_{11}NO_3$	121.1	100 g 500 g 1 kg	103133

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Ultra Pure Reagents

For critical, sensitive, demanding research where even a very minute amount of contaminant can potentially wreak havoc, MP Biomedicals Ultra Pure Reagents can provide the high quality you require. Using special purification steps, such as multiple re-distillations and recrystallizations (up to 5X), MP Bio purifies these reagents to uncommonly stringent specifications, making these products truly Ultra Pure. For example, during gel electrophoresis, it is often difficult to work at lower temperatures and pH because of marked precipitation when using sodium dodecyl sulfate (SDS). MP Bio solves this problem with our Ultra Pure lithium dodecyl sulfate (LDS), which exhibits greater solubility than SDS at lower temperatures, while maintaining similar detergency and wetting ability. Substitution of Ultra Pure LDS for SDS has been shown to result in greater resolution for certain proteins. Similarly, metallic and anionic contaminants, even in minute amounts, can shut down or block enzymatic proteins, resulting in poor yields and/or incorrect analytical and electrophoretic results. Use of Ultra Pure reagents often eliminates trace amounts of metallic contaminants and provides a better result. Remember, if it doesn't say "Ultra Pure", it probably isn't. With MP Bio Ultra Pure reagents, no finer quality products are available anywhere, at any price.

Name	Description	Pack Size	Cat. No.
ACES, Ultra Pure	$C_4H_{10}N_2O_4S$ MW 182.2. Purity: >99%. A zwitterionic buffer with useful pH range of 6.1–7.5. Used as an efficient separator (pH gradient of less than 1 pH unit) in the resolution of protein systems by IEF. Improves phenotyping of a1-antitrypsin by isoelectric focusing on agarose gels.	5 g	193973
		25 g	
		250 g	
Acrylamide, Ultra Pure	C_3H_5NO MW 71.1. Purity >99.9%. Acrylic acid content: < 0.001%. Super pure monomer for preparation of polyacrylamide gels for sensitive PAGE applications.	100 g	814320
		250 g	814323
		500 g	814326
		1 kg	814329
Ammonium Sulfate, Ultra Pure	$(NH_4)_2SO_4$ MW 132.2. Purity: ≥ 99%. A widely used reagent in molecular biology for the isolation and purification of enzymes and proteins. It is used for the precipitation or fractionation of proteins and for purification of antibodies. Ammonium sulfate is used in long PCR buffer, in PCR lysis solution, and in second strand cDNA synthesis.	50 g	808210
		500 g	821945
		1 kg	808211
		5 kg	808229
Calcium Chloride, Ultra Pure	Dihydrate. $CaCl_2 \cdot H_2O$ MW 147.0. Purity: ≥99%. Calcium chloride is a commonly used reagent in biochemistry. It is used in the preparation and transformation of competent *E. coli* and in the transfection of eukaryotic cells with either plasmid DNA or high MW genomic DNA.	100 g	193818
		500 g	
		1 kg	
Cesium Chloride, Ultra Pure	CsCl MW 168.36. Purity: >99.999%. Cesium chloride is typically used for density gradient work and for the purification of virus/phage, nucleic acids and nucleoproteins. It is used for the preparation of electrically conducting glasses, used to make solutions for the separation of RNA from DNA by density gradient centrifugation.	100 g	813061
		500 g	813063
		1 kg	813069
Cesium Chloride, Ultra Pure	CsCl MW 168.36. Purity: ≥99.999%. Cesium chloride is typically used for density gradient work and for the purification of virus/phage, nucleic acids and nucleoproteins. It is used for the preparation of electrically conducting glasses, used to make solutions for the separation of RNA from DNA by density gradient centrifugation.	5 g	150589
		25 g	
		100 g	
		500 g	
		1 kg	

Name	Description	Pack Size	Cat. No.
Ethidium Bromide, Ultra Pure	Purity: ≥98%. This Ultra Pure EtBr is ideal for fluorometric detection of double stranded nucleic acids in PAGE or agarose gels and in the separation of high MW DNAs. It also acts as an RNA polymerase inhibitor.	250 mg	823061
		1 g	823062
		5 g	823063
		25 g	823064
Ethidium Bromide Solution, Ultra Pure	A 10 mg/mL easy-to-use solution of ethidium bromide in specially filtered, deionized water, which is excellent for nucleic acid electrophoresis and purification applications. It eliminates the dust hazard associated with powdered ethidium bromide and saves time spent on weighing and mixing.	10 mL	802511
Formamide, Ultra Pure	Purity: 99.9%. For nucleic acid hybridization and sequencing in denaturing polyacrylamide gels. Typically needs to be deionized with an ion-exchange resin prior to use to eliminate formic acid that can breakdown nucleic acids.	100 g	800685
		500 g	800686
Glycerol, Ultra Pure	$C_3H_8O_3$ M.W. 92.09. Purity: ≥99.5%. Glycerol is used both in sample preparation and gel formation for polyacrylamide gel electrophoresis. It increases sample density to layer the sample at the bottom of the sample well. It is used in the concentration and storage of enzymes and also prevents back-diffusion of protein samples into the buffer.	500 mL	800687
		1 L	800688
		4 L	800689
Guanidine Hydrochloride, Ultra Pure	Purity: > 99%. Guanidine Hydrochloride is a strong chaotropic agent useful for the denaturation and subsequent refolding of proteins. It is used in the isolation of RNA to dissociate the nucleoprotein into its nucleic acid and protein moieties, and is an inhibitor of RNase.	100 g	820512
		500 g	820539
		1 kg	820540
Guanidine Hydrochloride, Ultra Pure	Purity: ≥ 99.5%. This strong denaturant can solubilize insoluble or denatured proteins, such as inclusion bodies. Highly concentrated (6 - 8 M) Guanidine HCl solutions are used to denature native globular proteins, presumably by disrupting the hydrogen bonds that hold the protein in its unique structure.	25 g	105696
		100 g	
		500 g	
		1 kg	
		5 kg	
N-Lauroylsarcosine sodium salt, Ultra Pure	Purity: ≥97%. An anionic detergent useful in the cell lysis process of RNA purification. Ideal for solubilizing membrane proteins prior to electrophoresis.	50 g	194009
		100 g	
		500 g	
Lithium dodecylsulfate, Ultra Pure	(LDS). Purity: >99%. Detergent for solubilizing proteins for electrophoresis. Demonstrates greater solubility than SDS at lower temperatures, while maintaining similar detergency and wetting ability.	5 g	800752
		25 g	800753
N,N'-Methylene-bis-acrylamide, Ultra Pure	Purity: 99.9%. A highly purified bisacrylamide for crosslinking with acrylamide to make superior PAGE gels for critical electrophoresis applications. May be used in UV scanning gels due to its optical clarity. Acrylic acid content: <0.02%	5 g	800172
		10 g	800171
		25 g	800706
		50 g	800173
		250 g	800175
		1 kg	800178

Ultra Pure Reagents

Name	Description	Pack Size	Cat. No.
Phenol, Ultra Pure, 99%	For the extraction of nucleic acids and to solubilize and denature proteins. Typically used in a mixture of phenol and buffered aqueous solution, proteins are denatured and collected at the interphase, while most nucleic acids remain in the aqueous phase.	100 g	800672
		500 g	818048
		1 kg	800673
Sodium dodecylsulfate, Ultra Pure	Purity: ≥99%. An anionic surfactant that denatures and solubilizes proteins for electrophoresis. Also useful as an aid in cell lysis during DNA extraction, and for dispersing and suspending nanotubes.	25 g	811033
		50 g	811036
		100 g	811034
		500 g	811032
		1 kg	811030
Sucrose, Ultra Pure	$C_{12}H_{22}O_{11}$ M.W. 342.30. Purity: 99.9%. DNase and RNase-free. Used for preparation of sucrose gradients for purification of proteins and RNAs.	100 g	802536
		500 g	821713
		1 kg	821721
Tris(hydroxymethyl) aminomethane, Ultra Pure, 99.95%	(TRIS base). Purity: 99.95%. Widely used zwitterionic Good's buffer for preparation of many different electrophoresis buffers. pK_a = 8.06 at 20°C.	100 g	103133
		250 g	
		500 g	
		1 kg	
		5 kg	
Tris(hydroxymethyl) aminomethane, Ultra Pure, 99.9%	(TRIS base). Purity: 99.9%. Widely used zwitterionic Good's buffer for preparation of many different electrophoresis buffers. pK_a = 8.06 at 20°C.	50 g	819619
		100 g	821557
		500 g	819620
		1 kg	819623
		5 kg	819638
Urea, Ultrapure, 99%	Purity: 99%. A high purity protein denaturant frequently added to buffers and solutions used in protein research.	1 lb	105695
		5 lb	
Urea, Ultra Pure	CH_4N_2O M.W. 60.06. Purity: ≥99%. An ultra pure reagent suitable for use as a protein denaturant. Urea is commonly used to solubilize and denature proteins for denaturing isoelectric focusing and two-dimensional electrophoresis and in acetic acid-urea PAGE gels. Urea is typically used at a concentration of 8 M for protein denaturation or solubilization. A final concentration of 5 M urea is commonly used in molecular biology for sequencing gels.	1 lb	821519
		5 lb	821527
		25 lb	821532
		1 kg	821528
		5 kg	821530
		10 kg	821858
		25 kg	821531

Make Your Own Buffer Solutions Cookbook

The following are recommended recipes for preparing the most commonly used buffers in electrophoresis applications. Whenever possible, MP Bio strongly recommends using Ultra Pure reagents and water when preparing them.

Tris-Glycine Native Running Buffer

Format:	Shelf-life:	pH:
500 mL of 10X solution	**1 year** at room temperature	**8.3**

Component	1X Concentration	Quantity for 10X solution
Tris	25 mM	29.0 g
Glycine	192 mM	144.0 g
Deionized water (ultra pure)	—	to 1.0 L

Tris-Glycine Native Sample Buffer

Format:	Shelf-life:	pH:
20 mL of 2X solution	**1 year** at 4°C	**8.6**

Component	1X Concentration	Quantity for 2X solution
Tris HCL	100 mM	4 mL of a 0.5 M sol.
Glycerol	10%	2 mL
Bromophenol Blue	0.0025%	0.5 mL of a 1% sol
Deionized water (ultra pure)	—	to 10.0 mL

Tris-Glycine Native Transfer Buffer

Format:	Shelf-life:	pH:
500 mL of 25X solution	**1 year** at room temperature	**8.3**

Component	1X Concentration	Quantity for 25X solution
Tris	12 mM	18.2 g
Glycine	96 mM	90.0 g
Deionized water (ultra pure)	—	to 500 mL

Tris-Glycine-SDS Running Buffer

Format:	Shelf-life:	pH:
500 mL of 10X solution	**1 year** at room temperature	**8.3**

Component	1X Concentration	Quantity for 10X solution
Tris	25 mM	29.0 g
Glycine	192 mM	144.0 g
SDS	0.1%	10.0 g
Deionized water (ultra pure)	—	to 1.0 L

Tris-Glycine-SDS Sample Buffer

Format:	Shelf-life:	pH:
20 mL of 2X solution	**1 year** at 4°C	**6.8**

Component	1X Concentration	Quantity for 2X solution
Tris HCl	63 mM	2.5 mL of a 0.5 M sol.
Glycerol	10%	2 mL
SDS	2%	4 mL of a 10% (wv) Sol.
Bromophenol Blue	0.0025%	0.5 mL of a 1% Sol.
Deionized water (ultra pure)	—	to 10.0 mL

Tris-Tricine-SDS Running Buffer

Format:	Shelf-life:	pH:
500 mL of 10X solution	**1 year** at room temperature	**8.3**

Component	1X Concentration	Quantity for 10X solution
Tris pH 8.3	100 mM	121.0 g
Tricine	100 mM	179.0 g
SDS	0.1%	10.0 g
Deionized water (ultra pure)	—	to 1.0 L

Tris-Tricine-SDS Sample Buffer

Format:	Shelf-life:	pH:
20 mL of 2X solution	**1 year** at 4°C	**8.45**

Component	1X Concentration	Quantity for 2X solution
Tris HCl, pH 8.45	450 mM	3 mL of a 3.0 M sol.
Glycerol	12%	2.4 mL
SDS	4%	0.8 g
Coomassie Blue G250	0.0025%	0.5 mL of a 1% sol.
Phenol Red	0.0025%	0.5 mL of a 1% sol.
Deionized water (pure water)	—	to 10.0 mL

TBE Running Buffer

Format:	Shelf-life:	pH:
1000 mL of 5X solution	**1 year** at room temperature	**8.3**

Component	1X Concentration	Quantity for 5X solution
Tris	89 mM	54.0 g
Boric acid	89 mM	27.5 g
EDTA (free acid)	2 mM	2.9 g
Deionized water (ultra pure)	—	to 1.0 L

TBE Sample Buffer

Format:	Shelf-life:
10 mL of 6X solution	**1 year** at 4°C

Component	1X Concentration	Quantity for 6X solution
Tris	45 mM	6 mL of 5X TBE running buffer
Boric acid	45 mM	—
EDTA (free acid)	1 mM	—
Glycerol	5.3%	3.2 mL
Bromophenol Blue	0.005%	0.3 mL of a 1% Sol.
Xylene Cyanol	0.005%	0.3 mL of a 1% Sol.
Deionized water (ultra pure)	—	to 10.0 mL

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