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Biological Buffers

Application

Many biochemical processes are markedly impaired by even small changes in the concentrations of free H^+ ions. It is therefore usually necessary to stabilise the H^+ concentration in vitro by adding a suitable buffer to the medium, without, however, affecting the functioning of the system under investigation.
A buffer keeps the pH value of a solution constant by taking up protons that are released during reactions, or by releasing protons when they are consumed by reactions.
This handout summarizes the most commonly used buffer substances and their respective physical and chemical properties.

Keywords

- Buffer characteristics
- Useful pH range
- Preparing buffer solutions
- Common buffer solutions

Practical tips – Preparing buffer solutions

Recommendations for the setting of the pH value of a buffer and storage conditions

Temperature

Depending on the buffer substance, its pH may vary with temperature. It is therefore advisable, as far as possible, to set the pH at the working temperature to be used for the investigation. For instance the physiological pH value for most mammalian cells at 37°C is between 7.0 and 7.5. The temperature dependence of a buffer system is expressed as *d(pKa)/dT*, which describes the change of the pK_a at an increase of temperature by 1°C.

Titration

1. Generally, the pH value is set using NaOH/KOH or HCl. Slow addition of a strong acid or base whilst stirring vigorously avoids local high concentrations of H^+ or OH^- ions. If this is not done, the buffer substances may undergo chemical changes that inactivate them or modify them so that they have an inhibitory action (Ellis & Morrison 1982).
2. Under stirring CO_2 dissolves in the solution. Stir solutions gently for precise measurements of the pH value.
3. If a buffer is available in the protonised form (acid) and the non-protonised form (base), the pH value can also be set by mixing the two substances.
4. Setting of the ionic strength of a buffer solution (if necessary) should be done in the same way as the setting of the pH value when selecting the electrolyte, since this increases depending on the electrolyte used.
5. If other components are added to the buffer (e.g. EDTA, DTT, Mg^{2+}, β-Mercaptoethanol) changes in the pH should also be considered and pH should be retested.
6. In the presence of divalent metal ions carbonate or phosphate buffers may form precipitates.

How can microbial contamination of buffer solutions be prevented?

1. Sterilize solutions by filtration through a 0.22 µm filter unit or by autoclaving.
2. Addition of 0.02 % (3 mM) sodium azide.
3. Storage at +4°C.
4. Prepare high-concentration stock solutions.

Code	Description	Buffer substance (short name)	Buffer substance name	pKa (25°C, 100 mM)	Effective pH range	autoclavable	Temperature dependence [d(pKa)/dT]	compatibility with protein assays (concentration limits)			Comments, effects in different assays
								BCA	Lowry	Bradford	
A1060	ACES for buffer solutions	ACES	N-(2-Acetamido)-2-aminoethanesulfonic acid	6.78	6.1 - 7.5	+	-0.020		+		significant absorption of UV light at 230 nm; binds Cu^{2+}
A0838	2-Amino-2-Methyl-1-Propanol for buffer solutions	AMP	2-Amino-2-methyl-1-propanol	9.69	8.7 - 10.4	n.a.	-0.032				
A1062	BES for buffer solutions	BES	N,N-Bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid	7.09	6.4 - 7.8	+	-0.016	-	+		binds Cu^{2+}
A1024	Bicine for buffer solutions	Bicine	N,N-Bis-(2-hydroxyethyl)-glycine	8.26	7.6 - 9.0	+	-0.018	+	+		slowly oxidized by ferricyanide; strongly binds Cu^{2+}
A1025	Bis-Tris for buffer solutions	BIS-Tris	[Bis-(2-hydroxyethyl)-imino]-tris-(hydroxymethylmethane)	6.46	5.8 - 7.2	+	-0.017	+			substitute for cacodylate. May be autoclaved or treated with DEPC
A1135	Bis-Tris-Propane for buffer solutions	BIS-Tris-Propane	1,3-Bis[tris(hydroxymethyl)-methylamino] propane	6.80	6.3 - 9.5	+					
A2940	Boric Acid for molecular biology	Boric acid		9.23 (pK_1), 12.74 (pK_2), 13.80 (pK_3)	8.5 - 10.2	+	-0.008 (pK_1)	(10 mM)			forms covalent complexes with mono- and oligosaccharides, ribose subunits of nucleic acids, pyridine nucleotides, glycerol
A2140	Cacodylic Acid Sodium Salt 3-hydrate BioChemica	Cacodylate	Dimethylarsinic acid	6.27	5.0 - 7.4	+					very toxic; nowadays usually replaced by MES
A3900	Sodium Carbonate anhydrous BioChemica	Carbonate	Sodium carbonate	6.35 (pK_1), 10.3 (pK_2)	6.0 - 8.0, 9.5 - 11.1		-0.0055 (pK_1), -0.009 (pK_2)				limited solubility; needs closed system, since in equilibrium with CO_2
A3901	tri-Sodium Citrate 2-hydrate BioChemica	Citrate	Salt of citric acid	3.13 (pK_1), 4.76 (pK_2), 6.40 (pK_3)	2.2 - 6.5, 3.0 - 6.2, 5.5 - 7.2	+		(<1 mM)	(2.5 mM)	(50 mM)	binds to some proteins, forms complexes with metals; often replaced by MES
A1067	Glycine for molecular biology	Glycine		2.35 (pK_1), 9.78 (pK_2)	2.2 - 3.6, 8.8 - 10.6	+	-0.0025 (pK_2)	(1 M)	(2.5 mM)	(0.1 M)	interferes with Bradford protein assay
A1069 A3724 A1070	HEPES for buffer solutions HEPES for molecular biology HEPES Sodium Salt for buffer solutions	HEPES	N-(2-Hydroxyethyl)-piperazine-N'-ethanesulfonic acid	7.48	6.8 - 8.2	+*	-0.014	-	+		can form radicals, not suitable for redox studies.
A1072	HEPPSO for buffer solutions	HEPPSO	N-(2-Hydroxyethyl)-piperazine-N'-2-hydroxypropanesulfonic acid	7.85	7.1 - 8.5	n.a.	-0.010	-	+		can form radicals, not suitable for redox studies
A1073 A1378	Imidazole for buffer solutions Imidazole for molecular biology	Imidazole		6.95	6.2 - 7.8	+*	-0.020				forms complexes with divalent metal cations, relatively unstable
A1074 A4730	MES 1-hydrate for buffer solutions MES 1-hydrate for molecular biology	MES	2-(N-Morpholino)-ethanesulfonic acid	6.10	5.5 - 6.7	+	-0.011	-	+		substitute for cacodylate
A1076 A2947 A1077	MOPS for buffer solutions MOPS for molecular biology MOPS Sodium Salt for buffer solutions	MOPS	3-(N-Morpholino)-propanesulfonic acid	7.14	6.5 - 7.9	+*	-0.011	-	+		partly degraded on autoclaving in the presence of glucose; negligible metal ion binding. May be autoclaved (change in colour does not influence buffer capacity)
A3905	di-Sodium hydrogen phosphate dihydrate BioChemica	Phosphate	Salt of phosphoric acid	2.15 (pK_1), 7.20 (pK_2), 12.33 (pK_3)	1.7 - 2.9, 5.8 - 8.0	+	0.0044 (pK_1), -0.0028 (pK_2), -0.026 (pK_3)	(250 µM)	(250 mM)	(2 M)	substrate/inhibitor of various enzymes (inhibits many kinases and dehydrogenases, enzymes with phosphate esters as substrate; inhibits carboxypeptidase, fumarase, urease); precipitates/ binds bivalent cations; pK increases on dilution
A1079	PIPES for buffer solutions	PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)	6.76	6.1 - 7.5	+	-0.0085	-	+		can form radicals, not suitable for redox studies. May be treated with DEPC
A1084	TES for buffer solutions	TES	2-[Tris(hydroxymethyl)-methylamino]-ethanesulfonic acid	7.40	6.8 - 8.2	+	-0.020	-	+		binds Cu^{2+}
A1085 A3954	Tricine BioChemica Tricine for molecular biology	Tricine	N-[Tris(hydroxymethyl)-methyl]-glycine	8.05	7.4 - 8.8	+	-0.021	+	+		strongly binds Cu^{2+}; addition of Cu^{2+} in the Lowry assay enables it to be used; is photooxidised by flavines; substitute for barbital (Veronal)
A1379 A1086 A2264	Tris for buffer solutions Tris ultrapure Tris for molecular biology	Tris	Tris(hydroxymethyl)-aminomethane	8.06	7.5 - 9.0	+	-0.028	(0.1 M)	(250 mM)	(2 M)	high degree of temperature-sensitivity; pH decreases by 0.1 unit with each 10-fold dilution; inactivates DEPC, can form Schiff's bases with aldehydes/ ketones, as it is a primary amine; is involved in some enzymatic reactions (e.g. alkaline phosphatase); toxic for many cells, since it penetrates cells due to its relatively good fat solubility

*Preferred method of sterilization is filtration rather than autoclaving for HEPES, Imidazole, MOPS, TEA and others.

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Recipes for commonly used buffer solutions and stocks

To prepare 1 litre of buffer solution dissolve ingredients in approx. 800 ml of deionised water, adjust pH value, add deionised water to 1000 ml final volume, and sterilize if desired.

HeBS transfection buffer (2X)

HEPES	11.9 g/L	(0.050 M)
Na_2HPO_4	0.21 g/L	(1.5 mM)
NaCl	16.4 g/L	(0.280 M)

exactly (!) adjust pH 7.1 with NaOH; filter sterilize; store aliquots at –20°C

MOPS buffer (1X)

MOPS	41.85 g/L	(0.2 M)
Na-acetate	41.02 g/L	(0.5 M)
EDTA-Na_2·$2H_2O$	3.72 g/L	(0.01 M)

adjust pH 7.0; filter sterilize, do not autoclave; MOPS solutions turn dark upon heating; store in the dark and discard if it turns yellow

PBS Phosphate-buffered saline (10X)

KH_2PO_4	2.4 g/L	(0.018 M)
Na_2HPO_4	14.4 g/L	(0.101 M)
NaCl	80 g/L	(1.369 M)
KCl	2 g/L	(0.027 M)

pH (20°C): 7.4; autoclave

SDS-Tris-Glycine buffer (10X) – "Laemmli" Buffer

Cat. No. A1415

Tris	30.29 g/L	(0.25 M)
Glycine	144.13 g/L	(1.92 M)
SDS	10 g/L	(1 %)

pH ~8.3; do not adjust pH value with additional ions; slight deviations may be tolerated

SSC Buffer (20X)

Cat. No. A1396

tri-Na citrate ·$2H_2O$	88.23 g/L	(0.3 M)
NaCl	175.32 g/L	(3 M)

adjust pH to 7.0; autoclave

TAE buffer (50X)

Cat. No. A4686

Tris	242.30 g/L	(2 M)
EDTA-Na_2·$2H_2O$	18.6 g/L	(0.05 M)
Acetic acid glac.	60.05 g/L	(1 M)

adjust pH to 8.5

TAE buffer (10X)

Cat. No. A3945

Tris	107.81 g/L	(0.89 M)
Boric acid	55.03 g/L	(0.89 M)
EDTA-Na_2·$2H_2O$	7.44 g/L	(0.02 M)

adjust pH to 8.3; autoclave

TBS buffer (1X, Tris buffered saline) recipe 1

Tris	3 g/L	(0.025 M)
KCl	0.2 g/L	(2.68 mM)
NaCl	8 g/L	(0.137 M)
Phenol red (optional pH indicator)	0.015 g/L	

Adjust pH to 7.4; filter sterilize or autoclave

TBS buffer (1X, Tris buffered saline) recipe 2

Tris-Cl	15.76 g/L	(0.1 M)
NaCl	8.77 g/L	(0.15 M)

adjust pH to 7.5; autoclave

TE buffer (100X)

Tris	121.14 g/L	(1 M)
EDTA-Na_2·$2H_2O$	37.22 g/L	(0.1 M)

adjust pH to 8.0; pH values 7.0, 7.4, 7.5 or 7.6 are also commonly used; autoclave

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