infoPoint

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+}, ß-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.

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PanReac AppliChem ITW Reagents

Product number	Product name	CAS number	Pack sizes	Buffer substance (short name)	Buffer substance name	pKa (25°C, 100 mM)	Effective pH range	Auto-clavable	Temperature dependence [d(pKa)/dT]	Compatibility with protein assays (concentration limits)			Comments, effects in different assays
										BCA	Lowry	Bradford	
A1060	ACES for buffer solutions	7365-82-4	1 kg, 10 kg	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	124-68-5	4 kg	AMP	2-Amino-2-methyl-1-propanol	9.69	8.7 - 10.4	n.a.	-0.032				
A1025	Bis-Tris for buffer solutions	6976-37-0	250 g, 500 g, 1 kg	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
131015	Boric Acid for analysis, ACS, ISO	10043-35-3	500 g, 1 kg, 5 kg	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
A1067	Glycine for molecular biology	56-40-6	1 kg, 5 kg	Glycine		2.35 (pK_1), 9.78 ($p K_2$)	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	HEPES for buffer solutions	7365-45-9	100 g, 500 g, 1kg, 5 kg	HEPES	N-(2-Hydroxyethyl)-piperazine-N'-ethanesulfonic acid	7.48	6.8 - 8.2	+*	-0.014	–	+		Can form radicals, not suitable for redox studies
A3724	HEPES for molecular biology		250 g, 500 g, 1 kg										
A1072	HEPPSO for buffer solutions	68399-78-0	100 g	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
A1074	MES 1-hydrate for buffer solutions	145224-94-8	250 g, 1 kg	MES	2-(N-Morpholino)-ethanesulfonic acid	6.10	5.5 - 6.7	+	-0.011	–	+		Substitute for cacodylate
A1076	MOPS for buffer solutions	1132-61-2	250 g, 1 kg, 5 kg	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)
A2947	MOPS for molecular biology		500 g, 1 kg										
A1079	PIPES for buffer solutions	5625-37-6	500 g	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	7365-44-8	1 kg	TES	2-[Tris(hydroxymethyl)-methylamino]-ethanesulfonic acid	7.40	6.8 - 8.2	+	-0.020	–	+		Binds Cu^{2+}
A1085	Tricine BioChemica	5704-04-1	250 g, 1 kg, 5 kg	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 photooxidized by flavines; substitute for barbital (Veronal)
A1379	Tris for buffer solutions	77-86-1	1 kg, 5 kg	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
A1086	Tris for analysis, ACS, ultrapure		1 kg, 5 kg, 10 kg										
A2264	Tris for molecular biology		500 g, 1 kg, 5 kg										

*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) (10X) "Laemmli" buffer

Product number 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)

Product number 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)

Product number A4686

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

Adjust pH to 8.5

TBE buffer (10X)

Product number 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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