AppliCations

No.2

Biological Buffers

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 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 respective physical and chemical properties.

AppliChem

Keywords

chemical properties

usefull pH range

buffer preparation

Practical Tips – Preparing Buffer Solutions

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

1. 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 $d(pK_a)/dT$, which describes the change of the pK_a at an increase of temperature by 1°C.

2. Titration

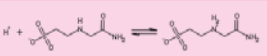
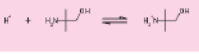
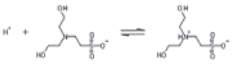
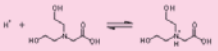
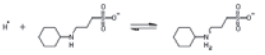
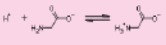
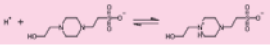
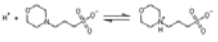
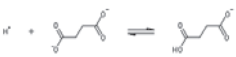
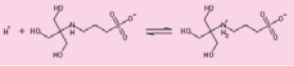
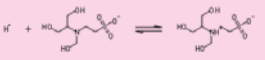
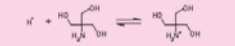
(i) Generally, the pH value is set using NaOH/KOH or HCl. Slow addition of a strong acid or base whilst stirring vigorously avoids local high concentrations of H^+ or OH^- ions. If this is not done, the buffer substances may undergo chemical changes that inactivate them or modify them so that they have an inhibitory action (Ellis & Morrison 1982). (ii) Under stirring CO_2 dissolves in the solution. Stir solutions gently for precise measurements of the pH value. (iii) 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. (iv) 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. (v) 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. (vi) In the presence of divalent metal ions carbonate or phosphate buffers may form precipitates .

3. How can microbial contamination of buffer solutions be prevented?

(i) Sterilization by filtration through a 0.22 µm filter unit or by autoclaving. (ii) Addition of 0.02 % (3 mM) sodium azide. (iii) Storage at +4°C. (iv) High-concentration stock solutions.

Catalog No.	Description	Name	pK_a (25°C, 100 mM)	Effective pH range	autoclavable
A1060, A3986[mb]	ACES	N-(2-Acetamido)-2-aminoethanesulfonic acid	6.78	6.1 - 7.5	+
A1045[Na]	Acetate	Salt of acetic acid	4.76	3.6 - 5.6	+
A1061	ADA	N-(2-Acetamido)-iminodiacetic acid	6.59	6.0 - 7.2	n.a.
A3654[bc]	Ammonia		9.25	8.8 - 9.9	–
A0838	AMP	2-Amino-2-methyl-1-propanol	9.69	8.7 - 1 0.4	n.a.
A1158	AMPD	2-Amino-2-methyl-1,3-propanediol, Ammediol	8.80	7.8 - 9.7	+
A1075	AMPSO	N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropane-sulfonic acid	9.00	8.3 - 9.7	+
A1062, A4637[mb]	BES	N,N-Bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid	7.09	6.4 - 7.8	+
A1024, A3988[mb]	Bicine	N,N-Bis-(2-hydroxyethyl)-glycine	8.26	7.6 - 9.0	+
A1025	BIS-Tris	[Bis-(2-hydroxyethyl)-imino]-tris-(hydroxymethylmethane)	6.46	5.8 - 7.2	+
A1135	BIS-Tris-Propane	1,3-Bis[tris(hydroxymethyl)-methylamino]propane	6.80	6.3 - 9.5	+
A0768 , A1097[bc], A2940[mb]	Boric acid		9.23 (pK_1), 12.74 (pK_2), 13.80 (pK_3)	8.5 - 1 0.2	+
A1497[bc], A2140[Na]	Cacodylate	Dimethylarsinic acid	6.27	5.0 - 7.4	+
A1063, A1136[Na]	CAPS	3-(Cyclohexylamino)-propanesulfonic acid	10.40	9.7 - 11 .1	+
A1064	CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid	9.60	8.9 - 1 0.3	+
A3900[bc], A1940[bc]	Carbonate	Sodium carbonate	6.35 (pK_1), 10.3 (pK_2)	6.0 - 8.0, 9.5 - 11.1	
A1065	CHES	2-(N-Cyclohexylamino)-ethanesulfonic acid	9.50	8.6 - 10.0	
A3901[Na]	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	+
A1066	DIPSO	3-[N-Bis(hydroxyethyl)amino]-2-hydroxypropanesulfonic acid	7.52	7.0 - 8.2	n.a.
A3858[bc]	Formate	Salt of formic acid	3.75	3.0 - 4.5	+
A3707[bc], A3741[cc], A1067[mb]	Glycine		2.35 (pK_1), 9.78 (pK_2)	2.2 - 3.6, 8.8 - 10.6	+
A1068, A4753[cc], A1137[HCl]	Glycylglycine		3.14 (pK_1), 8.25 (pK_2)	2.5 - 3.8, 7.5 - 8.9	+
A1069, A3268[cc], A3724[mb], A1070[Na]	HEPES	N-(2-Hydroxyethyl)-piperazine-N'-ethanesulfonic acid	7.48	6.8 - 8.2	+
A1071	HEPPS, EPPS	N-(2-Hydroxyethyl)-piperazine-N'-3-propanesulfonic acid	8.00	7.6 - 8.6	+
A1072	HEPPSO	N-(2-Hydroxyethyl)-piperazine-N'-2-hydroxypropanesulfonic acid	7.85	7.1 - 8.5	n.a.
A1073, A1378[mb]	Imidazole		6.95	6.2 - 7.8	+
A3644[bc], A2130[bc], A1642[Na]	Malate	Salt of malic acid	3.40 (pK_1), 5.13 (pK_2)	2.7 - 4.2, 4.0 - 6.0	+
A1841, A4462[Na]	Maleate	Salt of maleic acid	1.97 (pK_1), 6.24 (pK_2)	1.2 - 2.6, 5.5 - 7.2	+
A1074, A4730[mb], A3101[Na]	MES	2-(N-Morpholino)-ethanesulfonic acid	6.10	5.5 - 6.7	+
A1076, A2947[mb], A1077[Na]	MOPS	3-(N-Morpholino)-propanesulfonic acid	7.14	6.5 - 7.9	+
A1078	MOPSO	3-(N-Morpholino)-2-hydroxypropanesulfonic acid	6.87	6.2 - 7.6	+
A2944[mb], A3902[bc], A4732[mb], A3905[bc]	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	+
A1079, A3495[mb], A1080[Na]	PIPES	Piperazine-N,N'-bis(2-ethanesulfonic acid)	6.76	6.1 - 7.5	+
A1081	POPSO	Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid)	7.78	7.2 - 8.5	+
A0776[pA]	Pyridine		5.23	4.9 - 5.9	–
A3627, A2136[Na]	Succinate	Salt of succinic acid	4.21 (pK_1), 5.64 (pK_2)	3.2 - 5.2, 5.5 - 6.5	+
A1082, A4740[mb]	TAPS	3-{[Tris(hydroxymethyl)-methyl]-amino}-propanesulfonic acid	8.40	7.7 - 9.1	+
A1083	TAPSO	3-[N-Tris(hydroxymethyl)-methylamino]-2-hydroxypropane-sulfonic acid	7.61	7.0 - 8.2	+
A1141[bc], A4235[cc]	Taurine	2-Aminoethanesulfonic acid, AES	9.06	8.4 - 9.6	(+)*
A1423[bc], A1424[HCl]	TEA	Triethanolamine	7.76	7.0 - 8.3	(+)*
A1084 , A3277[Na]	TES	2-[Tris(hydroxymethyl)-methylamino]-ethanesulfonic acid	7.40	6.8 - 8.2	+
A1085[bc], A4807[cc], A3954[mb]	Tricine	N-[Tris(hydroxymethyl)-methyl]-glycine	8.05	7.4 - 8.8	+
A1379, A1086[up], A2264[mb]	Tris	Tris(hydroxymethyl)-aminomethane	8.06	7.5 - 9.0	+

Buffer grade or [mb]: Molecular biology grade [bc]: BioChemica grade [cc]: Cell culture grade [pA]: Analytical grade [up]: ultrapure [Na]: Sodium salt [HCl]: Hydrochloride n.a.: data not available

Temperature dependence [d(pK$_a$)/dT]	compatibility with protein assays (concentration limits)			Comments, effects in different assays	Reaction $H^+ + A^- \rightleftharpoons HA$ or $H^+ + \equiv N \rightleftharpoons \equiv N^+H$
	BCA	Lowry	Bradford		
-0.020		+		significant absorption of UV light at 230 nm; binds Cu^{2+}	
0.0002	(0.2 M)		(0.6 M)		
-0.011	+	+		marked absorption in UV range below 260 nm; binds metal ions	
-0.031					
-0.032					
-0.029					
-0.016	-	+		binds Cu^{2+}	
-0.018	+	+		slowly oxidised by ferricyanide; strongly binds Cu^{2+}	
-0.017	+			substitute for cacodylate; may be autoclaved, may be treated with DEPC	
-0.008 (pK$_1$)	(10 mM)			forms covalent complexes with mono- and oligosaccharides, ribose subunits of nucleic acids, pyridine nucleotides, glycerol	
				very toxic; nowadays usually replaced by MES	
-0.009	-	+			
-0.0055 (pK$_1$), -0.009 (pK$_2$)				limited solubility; needs closed system, since in equilibrium with CO_2	
-0.011					
	(<1 mM)	(2.5 mM)	(50 mM)	binds to some proteins, forms complexes with metals; replaced by MES	
-0.015		+			
0.0					
-0.0025 (pK$_2$)	(1 M)	(2.5 mM)	(0.1 M)	interferes with Bradford protein assay	
-0.025				binds Cu^{2+}	
-0.014	-	+		can form radicals, not suitable for redox studies	
-0.015	-	+		can form radicals, not suitable for redox studies	
-0.010	-	+		can form radicals, not suitable for redox studies	
-0.020				forms complexes with Me^{2+}, relatively unstable	
				DL-Malic acid and L(-)-Malic acid available	
				absorbs in the UV range; replaced by MES or Bis-Tris	
-0.011	-	+		substitute for cacodylate	
-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)	
-0.015		+			
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	
-0.0085	-	+		can form radicals, not suitable for redox studies; may be autoclaved, may be treated with DEPC	
-0.013		+			
-0.014					
-0.0018 (pK$_1$), 0.0 (pK$_2$)					
0.018		+		does not bind Mg^{2+}, Ca^{2+}, Mn^{2+}, or Cu^{2+}; satisfactory for studies of electron transport	
-0.018		+			
-0.022					
-0.020					
-0.020	-	+		binds Cu^{2+}	
-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)	
-0.028	(0.1 M)	(250 mM)	(2 M)	high degree of temperature-sensitivity; pH decreases by 0.1 unit with each 10fold 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	

*: In the literature you will find information for several buffer substances that the preferred method of sterilization is filtration rather than autoclaving. This includes buffers such as HEPES, HEPPS, Imidazole, MOPS, Taurine, TEA and others.

Recipes for commonly used buffer solutions and stocks

To prepare 1 liter 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	11.90 g/L	50 mM
NaCl	16.40 g/L	280 mM
Na_2HPO_4	0.21 g/L	1.5 mM
exactly (!) adjust pH 7.1 with NaOH; filter sterilize; store aliquots at –20°C		

MOPS buffer (1X)		
MOPS	41.85 g/L	200 mM
Na-acetate	41.02 g/L	500 mM
EDTA · Na_2 · $2H_2O$	3.72 g/L	10 mM
adjust pH 7.0; filter sterilize or autoclave; MOPS solutions may turn dark upon heating; store in the dark and discard if it turns yellow		

PBS Phosphate-buffered saline (10X)		
KH_2PO_4	2.40 g/L	18 mM
Na_2HPO_4	14.40 g/L	101 mM
NaCl	80.00 g/L	1.369 M
KCl	2.00 g/L	27 mM
pH (20°C): 7.4; autoclave		

SDS-Tris-Glycine buffer (10X) - "Laemmli" buffer		**Cat. No. A1415**
Tris	30.29 g/L	250 mM
Glycine	144.13 g/L	1.920 M
SDS	10.00 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	300 mM
NaCl	175.32 g/L	3.0 M
adjust pH to 7.0; autoclave		

TAE buffer (50X)		**Cat. No. A4686**
Tris	242.30 g/L	2.0 M
EDTA-Na_2 · $2H_2O$	18.60 g/L	50 mM
Acetic acid glacial	60.05 g/L	1.0 mM
adjust pH to 8.5		

TBE buffer (10X)		**Cat. No. A3945**
Tris	107.81 g/L	890 mM
Boric acid	55.03 g/L	890 mM
EDTA-Na_2 · $2H_2O$	7.44 g/L	20 mM
adjust pH to 8.3; autoclave		

TBS buffer (1X, Tris-buffered saline) recipe 1		**Cat. No. A3836**
Tris	3.00 g/L	25 mM
KCl	0.20 g/L	2.68 mM
NaCl	8.00 g/L	137 mM
Phenol red (optional pH indicator)	0.015 g/L	0.042 mM
adjust pH to 7.4; filter sterilize		

TBS buffer (1X, Tris-buffered saline) recipe 2		
Tris-Cl	15.76 g/L	100 mM
NaCl	8.77 g/L	150 mM
adjust pH to 7.5; autoclave		

TE buffer (100X)		**Cat. No. A6554**
Tris	121.14 g/L	1.0 M
EDTA-Na_2 · $2H_2O$	37.22 g/L	100 mM
adjust pH to 7.5; pH values 7.0, 7.4, 7.6 or 8.0 are also commonly used; autoclave		

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