

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, Mg²⁺, 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 μ m filter unit or by autoclaving. (ii) Addition of 0.02 % (3 mM) sodium azide. (iii) Storage at +4°C. (iv) High-concentration stock solutions.

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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 A3707 ^{bc} , A3741 ^{cc} , A1067 ^{mb} Glycine Salt of formic acid 3.13 (pK ₁), 9.78 (pK ₂) 2.2 - 3.6, 8.8 - 10.6	+
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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 A3858 ^{bc} Formate Salt of formic acid 3.75 3.0 - 4.5 A3707 ^{bc} , A3741 ^{cc} , A1067 ^{mb} Glycine Salt of formic acid 3.14 (pk ₁), 8.25 (pk ₂) 2.2 - 3.6, 8.8 - 10.6	+
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 A3858 ^{bc} Salt of formic acid Salt of formic acid 3.75 3.0 - 4.5 A3707 ^{bc} , A3741 ^{cc} , A1067 ^{mb} Glycine Salt of formic acid 3.14 (pK ₁), 8.25 (pK ₂) 2.2 - 3.6, 8.8 - 10.6	
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 A3858 ^{bc} Formate Salt of formic acid 3.75 3.0 - 4.5 A3707 ^{bc} , A3741 ^{cc} , A1067 ^{mb} Glycine Salt of formic acid 3.14 (pK ₁), 8.25 (pK ₂) 2.2 - 3.6, 8.8 - 10.6	
A1066 DIPSO 3-[N-Bis(hydroxyethyl)amino]-2-hydroxypropanesulfonic acid 7.52 7.0 - 8.2 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 ^{HCI} Glycylglycine 3.14 (pK ₁), 8.25 (pK ₂) 2.5 - 3.8, 7.5 - 8.9	+
A3858 ^{bc} Formate Salt of formic acid 3.75 3.0 - 4.5 A3707 ^{bc} , A3741 ^{cc} , A1067 ^{mb} Glycine Salt of formic acid 2.35 (pK ₁), 9.78 (pK ₂) 2.2 - 3.6, 8.8 - 10.6 A1068, A4753 ^{cc} , A1137 ^{HCl} Glycylglycine Salt of formic acid 3.14 (pK ₁), 8.25 (pK ₂) 2.5 - 3.8, 7.5 - 8.9	n.a.
A1068, A4753°, A1137 ^{HCl} Glycylglycine 3.14 (pK ₁), 8.25 (pK ₂) 2.5 - 3.8, 7.5 - 8.9	+
	+
	+
A1069, A3268°, 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 7.85 7.1 - 8.5	n.a.
acid 6.95 A1073, A1378 ^{mb} Imidazole	+
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	+
$\frac{1}{10^{-6}, 42947^{mb}, A107^{Na}}$ 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	
A1082, A4740mbTAPS3-{[Tris(hydroxymethyl)-methyl]-amino}-propanesulfonic acid8.407.7 - 9.1	+
A1083 TAPSO 3-[N-Tris(hydroxymethyl)-methylamino]-2-hydroxypropane- sulfonic acid 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 depen-		lity with pro		Comments, effects in different assays	Reaction
dence [d(pK _a)/dT]	assays (concentration limits)		limits)		$H^+ + A^- \implies HA$ or $H^+ + N \equiv \implies H^+N \equiv$
	BCA	Lowry	Bradford		
-0.020		+		significant absorption of UV light at 230 nm; binds Cu ²⁺	H + Strike - Strike
					I all a construction of the second secon
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					
					н' + нун-р ^{он} 💳 нун-р ^{он}
-0.029					
-0.027					
-0.016	-	+		binds Cu ²⁺	он он
					، پېخر 💳 ، پېکر ۲ ۲
0.010					$u_{i} + \bigcup_{0,i}^{n_{i}} \bigcup_{0,i}^{n_{i}} \bigcup_{0,i}^{n_{i}} = \bigcup_{0,i}^{n_{i}} \bigcup_$
-0.018	+	+		slowly oxidised by ferricyanide; strongly binds Cu ²⁺	QH
					H' + HOWING HOWING
0.017				substitute for cacodylate; may be autoclaved, may be treated with DEPC	
-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,	[or] -
				pyridine nucleotides, glycerol	$H^{2} + \begin{bmatrix} OH \\ HO & OH \\ OH \end{bmatrix}^{-} = H_{0}O + H_{0}O + H_{0}OH$
				very toxic; nowadays usually replaced by MES	
-0.009	-	+		very toxic, nowadays usually replaced by with	" + [*]
					* • \ == \
					U n U n ₂
-0.0055 (pK ₁), -0.009 (pK ₂)				limited solubility; needs closed system, since in equilibrium with CO,	
·· · · · · · · · · · · · · · · · · · ·					₩ + Ĵ~ == Ĵ#
-0.011					
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	
-0.002) (pR ₂)	(1 1/1)	(2.) 11101)	(0.1 M)	interieres with bradioru protein assay	$\mu + \mu^{\mu} - \mu^{\mu} = \mu^{\mu} - \mu^{\mu}$
-0.025				binds Cu ²⁺	
-0.014	-	+		can form radicals, not suitable for redox studies	** +
-0.015 -0.010	-	+		can form radicals, not suitable for redox studies 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	$\omega \rightarrow Q \leftarrow Q$
					H + CHR = CHR
				DL-Malic acid and L(-)-Malic acid available	
				absorbs in the UV range; replaced by MES or Bis-Tris	н' + то-{С}-ст ≕ но-{С}-ст
-0.011	-	+		substitute for cacodylate	q + p(f) = p(f)
-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)	$*\cdot \cup \sim {} }{} }{} {} {} {} {} {} {} {} {} {} {} {} {} {} {} {} {} }{} {} {} {} {} {} {} {} {} {} {} {} {} {} {} {} {} {} {} }{} {} {} {} {} {} {} {} }{} {} {} {} {} {} {} {} {} }{} }{} {} {} {} }{} {} {} {} {} }{} {} {}} {} {}} {} {}} {} }{} {} }{} {} {} }{} {} }{} {}} {}} {} }{} }{} }{} {} }{$
-0.015		+			
0.0044 (pK ₁), -0.0028	(250 µM)	(250 mM)	(2 M)	substrate/inhibitor of various enzymes (inhibits many kinases and dehydrogenases, enzymes	0 0
(pK ₂), -0.026 (pK ₃)				with phosphate esters as substrate; inhibits carboxypeptidase, fumarase, urease); precipitates/	H' + TO HO HO HO -
-0.0085	-	+		binds bivalent cations; pK increases on dilution can form radicals, not suitable for redox studies; may be autoclaved, may be treated with DEPC	
-0.013		+		and the second states in the s	
-0.014					
-0.0018 (pK ₁), 0.0 (pK ₂)					$ \cdots \land \checkmark = \land \checkmark$
					$u' + u_{i} +$
0.018		+		does not bind Mg2+, Ca2+, Mn2+, or Cu2+; satisfactory for studies of electron transport	la so la so
					H, + HO HILL B = HO HE RO
-0.018		+			in the second se
-0.022					
-0.020 -0.020	-	+		binds Cu ²⁺	на на
					$H + Ha \int_{0}^{0H} f_0^{i} \Longrightarrow Ha \int_{0}^{0H} f_0^{i}$
					но но
-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	и + HQOH = HQOH
				involved in some enzymatic reactions (e.g. alkaline phosphatase); toxic for many cells, since it	нуй он нуй он
*. In the literature you will fi	nd informatio	n for several b	uffer substance	penetrates cells due to its relatively good fat solubility es that the preferred method of sterilization is filtration rather than autoclaving. This includes buffe	rs such as HEPES HEPPS Imidazola MOPS

*: 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 ₂ HPO 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
$EDTA \cdot Na_2 \cdot 2H_2O$	3.72 g/L	10 mM
adjust pH 7.0; filter sterilize or auto	,	lark upon heating;
store in the dark and discard if it tu	rns yellow	
PBS Phosphate-buffered saline	(10X)	
$\mathrm{KH}_2\mathrm{PO}_4$	2.40 g/L	18 mM
Na ₂ HPO 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 wi	th additional ions; slight deviation	is may be tolerated
SSC buffer (20X)		Cat. No.
		A1396
tri-Na citrate · 2H ₂ O	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 \cdot 2H ₂ O	18.60 g/L	50 mM
Acetic acid glacial	60.05 g/L	1.0 mM
adjust pH to 8.5	, i i i i i i i i i i i i i i i i i i i	
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 ₂ O	7.44 g/L	20 mM
adjust pH to 8.3; autoclave		
TBS buffer (1X, Tris-buffered saline	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 1	ised; autoclave

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