

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	Temperature dependence [d(pK _a)/dT]	compatibility with protein assays (concentration limits)			Comments, effects in different assays	Reaction H ⁺ + A ⁻ ⇌ HA or H ⁺ + N≡ ⇌ H ⁺ N≡
							BCA	Lowry	Bradford		
A1060, A3986 ^{mb}	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⁺	
A1045 ^{na}	Acetate	Salt of acetic acid	4.76	3.6 - 5.6	+	0.0002	(0.2 M)		(0.6 M)		
A1061	ADA	N-(2-Acetamido)-iminodiacetic acid	6.59	6.0 - 7.2	n.a.	-0.011	+	+		marked absorption in UV range below 260 nm; binds metal ions	
A3654 ^{bc}	Ammonia		9.25	8.8 - 9.9	-	-0.031					
A0838	AMP	2-Amino-2-methyl-1-propanol	9.69	8.7 - 10.4	n.a.	-0.052					
A1158	AMPD	2-Amino-2-methyl-1,3-propanediol, Ammediol	8.80	7.8 - 9.7	+	-0.029					
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	+	-0.016	-	+		binds Cu ²⁺	
A1024, A3988 ^{mb}	Bicine	N,N-Bis-(2-hydroxyethyl)-glycine	8.26	7.6 - 9.0	+	-0.018	+	+		slowly oxidised by ferricyanide; strongly binds Cu ²⁺	
A1025	BIS-Tris	[Bis-(2-hydroxyethyl)-imino]-tris-(hydroxymethylmethane)	6.46	5.8 - 7.2	+	-0.017	+			substitute for cacodylate; may be autoclaved, may be treated with DEPC	
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 - 10.2	+	-0.008 (pK ₁)	(10 mM)			forms covalent complexes with mono- and oligosaccharides, ribose subunits of nucleic acids, pyridine nucleotides, glycerol	
A1497 ^{bc} , A2140 ^{na}	Cacodylate	Dimethylarsinic acid	6.27	5.0 - 7.4	+					very toxic; nowadays usually replaced by MES	
A1063, A1136 ^{na}	CAPS	3-(Cyclohexylamino)-propanesulfonic acid	10.40	9.7 - 11.1	+	-0.009	-	+			
A1064	CAPSO	3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid	9.60	8.9 - 10.3	+					limited solubility; needs closed system, since in equilibrium with CO ₂	
A3900 ^{bc} , A1940 ^{bc}	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)					
A1065	CHES	2-(N-Cyclohexylamino)-ethanesulfonic acid	9.50	8.6 - 10.0		-0.011					
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	+		(<1 mM)	(2.5 mM)	(50 mM)	binds to some proteins, forms complexes with metals; replaced by MES	
A1066	DIPSO	3-[N-Bis(hydroxyethyl)amino]-2-hydroxypropanesulfonic acid	7.52	7.0 - 8.2	n.a.	-0.015		+			
A3858 ^{bc}	Formate	Salt of formic acid	3.75	3.0 - 4.5	+	0.0					
A3707 ^{bc} , A3741 ^{cc} , A1067 ^{mb}	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	
A1068, A4753 ^{cc} , A1137 ^{hcl}	Glycylglycine		3.14 (pK ₁), 8.25 (pK ₂)	2.5 - 3.8, 7.5 - 8.9	+	-0.025				binds Cu ²⁺	
A1069, A3268 ^{cc} , A3724 ^{mb} , A1070 ^{na}	HEPES	N-(2-Hydroxyethyl)-piperazine-N'-ethanesulfonic acid	7.48	6.8 - 8.2	+	-0.014	-	+		can form radicals, not suitable for redox studies	
A1071	HEPPS, EPPS	N-(2-Hydroxyethyl)-piperazine-N'-3-propanesulfonic acid	8.00	7.6 - 8.6	+	-0.015	-	+		can form radicals, not suitable for redox studies	
A1072	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 ^{mb}	Imidazole		6.95	6.2 - 7.8	+	-0.020				forms complexes with Me ²⁺ , relatively unstable	
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	+					DL-Malic acid and L(-)-Malic acid available	
A1841, A4462 ^{na}	Maleate	Salt of maleic acid	1.97 (pK ₁), 6.24 (pK ₂)	1.2 - 2.6, 5.5 - 7.2	+					absorbs in the UV range; replaced by MES or Bis-Tris	
A1074, A4730 ^{mb} , A3101 ^{na}	MES	2-(N-Morpholino)-ethanesulfonic acid	6.10	5.5 - 6.7	+	-0.011	-	+		substitute for cacodylate	
A1076, A2947 ^{mb} , A1077 ^{na}	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)	
A1078	MOPSO	3-(N-Morpholino)-2-hydroxypropanesulfonic acid	6.87	6.2 - 7.6	+	-0.015		+			
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	+	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, A3495 ^{mb} , A1080 ^{na}	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 autoclaved, may be treated with DEPC	
A1081	POPSO	Piperazine-N,N'-bis(2-hydroxypropanesulfonic acid)	7.78	7.2 - 8.5	+	-0.013		+			
A0776 ^{na}	Pyridine		5.23	4.9 - 5.9	-	-0.014					
A3627, A2136 ^{na}	Succinate	Salt of succinic acid	4.21 (pK ₁), 5.64 (pK ₂)	3.2 - 5.2, 5.5 - 6.5	+	-0.0018 (pK ₁), 0.0 (pK ₂)					
A1082, A4740 ^{mb}	TAPS	3-[[Tris(hydroxymethyl)-methyl]-amino]-propanesulfonic acid	8.40	7.7 - 9.1	+	0.018		+		does not bind Mg ²⁺ , Ca ²⁺ , Mn ²⁺ , or Cu ²⁺ ; satisfactory for studies of electron transport	
A1083	TAPSO	3-[N-Tris(hydroxymethyl)-methylamino]-2-hydroxypropane-sulfonic acid	7.61	7.0 - 8.2	+	-0.018		+			
A1141 ^{bc} , A4235 ^{cc}	Taurine	2-Aminoethanesulfonic acid, AES	9.06	8.4 - 9.6	(+)*	-0.022					
A1423 ^{bc} , A1424 ^{hcl}	TEA	Triethanolamine	7.76	7.0 - 8.3	(+)*	-0.020					
A1084, A3277 ^{na}	TES	2-[Tris(hydroxymethyl)-methylamino]-ethanesulfonic acid	7.40	6.8 - 8.2	+	-0.020	-	+		binds Cu ²⁺	
A1085 ^{bc} , A4807 ^{cc} , A3954 ^{mb}	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 ^{mb} , A2264 ^{mb}	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 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	

Buffer grade or ^{mb}: Molecular biology grade ^{bc}: BioChemica grade ^{cc}: Cell culture grade ^{na}: Analytical grade ^{mp}: ultrapure ^{na}: Sodium salt ^{hcl}: Hydrochloride n.a.: data not available

*: 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.

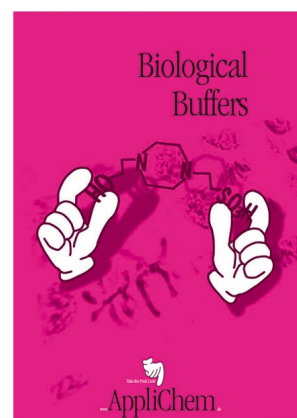
HeBS transfection buffer (2X)			TAE buffer (50X)			Cat. No.
HEPES	11.90 g/L	50 mM	Tris	242.30 g/L	2.0 M	A4686
NaCl	16.40 g/L	280 mM	EDTA-Na ₂ · 2H ₂ O	18.60 g/L	50 mM	
Na ₂ HPO ₄	0.21 g/L	1.5 mM	Acetic acid glacial	60.05 g/L	1.0 mM	
<i>exactly (!) adjust pH 7.1 with NaOH; filter sterilize; store aliquots at -20°C</i>			<i>adjust pH to 8.5</i>			
MOPS buffer (1X)			TBE buffer (10X)			Cat. No.
MOPS	41.85 g/L	200 mM	Tris	107.81 g/L	890 mM	A3945
Na-acetate	41.02 g/L	500 mM	Boric acid	55.03 g/L	890 mM	
EDTA · Na ₂ · 2H ₂ O	3.72 g/L	10 mM	EDTA-Na ₂ · 2H ₂ O	7.44 g/L	20 mM	
<i>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</i>			<i>adjust pH to 8.3; autoclave</i>			
PBS Phosphate-buffered saline (10X)			TBS buffer (1X, Tris-buffered saline) recipe 1			Cat. No.
KH ₂ PO ₄	2.40 g/L	18 mM	Tris	3.00 g/L	25 mM	A3836
Na ₂ HPO ₄	14.40 g/L	101 mM	KCl	0.20 g/L	2.68 mM	
NaCl	80.00 g/L	1.369 M	NaCl	8.00 g/L	137 mM	
KCl	2.00 g/L	27 mM	Phenol red (optional pH indicator)	0.015 g/L	0.042 mM	
<i>pH (20°C): 7.4; autoclave</i>			<i>adjust pH to 7.4; filter sterilize</i>			
SDS-Tris-Glycine buffer (10X) - "Laemmli" buffer			TBS buffer (1X, Tris-buffered saline) recipe 2			
		Cat. No.	Tris-Cl	15.76 g/L	100 mM	
Tris	30.29 g/L	A1415	NaCl	8.77 g/L	150 mM	
Glycine	144.13 g/L	1.920 M	<i>adjust pH to 7.5; autoclave</i>			
SDS	10.00 g/L	1 %	TE buffer (100X)			Cat. No.
<i>pH ~8.3; do not adjust pH value with additional ions; slight deviations may be tolerated</i>			A6554			
SSC buffer (20X)			Tris	121.14 g/L	1.0 M	
		Cat. No.	EDTA-Na ₂ · 2H ₂ O	37.22 g/L	100 mM	
tri-Na citrate · 2H ₂ O	88.23 g/L	A1396	<i>adjust pH to 7.5; pH values 7.0, 7.4, 7.6 or 8.0 are also commonly used; autoclave</i>			
NaCl	175.32 g/L	3.0 M				
<i>adjust pH to 7.0; autoclave</i>						

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