

Buffers and chemicals

A. Commonly Used Buffers

Buffer	Synonyms	pK	Molecular weight
Phosphate (pK ₁)		2.12	98.0, free acid
Glycine-HCl		2.34	111.53
Citrate (pK ₁)		3.14	192.1, free acid
Formate		3.75	68.0, Na salt
Carbonate (pK ₁)		3.76	106.6
Succinate (pK ₁)		4.19	118.1, free acid
Acetate		4.75	82.0, Na salt
Citrate (pK ₂)		4.76	214.1, Na salt
Succinate (pK ₂)		5.57	162.1, diNa salt
MES		6.15	195.2, hydrate
Carbonate (pK ₂)	Bicarbonate	6.36	84.0, bicarbonate
Citrate (pK ₃)		6.39	294.1, triNa salt, dihydrate
PIPES		6.8	302.4
ACES		6.9	182.2
MOPS		7.2	209.7
HEPES		7.55	238.3
Phosphate (pK ₂)		7.21	120.0, Na salt, monobasic
TES		7.7	229.2
Barbital	Barbitone Veronal	7.78	128.1, barbituric acid
Triethanolamine		7.8	149.2
TRICINE		8.15	179.2
TRIS		8.3	121.1
BICINE		8.35	163.2
Glycylglycine		8.4	132.1
Borate		9.24	201.2, Na tetraborate
CHES		9.5	207.3
Ethanolamine		9.5	61.1
Glycine-NaOH		9.6	97.1, Na salt hydrate
CAPS		10.4	221.3
Triethylamine		10.7	101.2
Phosphate (pK ₃)		12.3	141.9, Na salt, dibasic

B. Concentrations of Commercial Liquids

Compound	Molecular weight	Molarity	pH of dilute solutions		
			1 M	0.1 M	0.01 M
Acetic acid, glacial	60.05	17.4	2.4	2.9	3.4
Formic acid	46.02	23.4			
Hydrochloric acid, 38%	36.47	11.6	0.1	1.1	2.02
Nitric acid, 70%	63.02	16			
Phosphoric acid	98.0	18.1		1.5	
Sulfuric acid	98.08	18			
Ammonium hydroxide	35.0	14.8			
Ethanolamine, 99%	61.08	16.5			
Triethanolamine	101.19	7.16			
Formaldehyde, 37%	30.3	12.2			
Hydrogen peroxide, 30%	34.02	8.8			
-Mercaptoethanol	78.13	14.4			

C. Standard Buffer Solutions

1. 12.5 mM Borate buffer solutions, pH 8.1-9.0 at 25°C

Dissolve 0.476 g of borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) in 70 ml of distilled water and add x ml of 0.1 N HCl according to the pH required. Then Make up to 100 ml.

pH	x ml	pH	x ml
8.1	19.7	8.6	13.5
8.2	18.8	8.7	11.6
8.3	17.7	8.8	9.4
8.4	16.6	8.9	7.1
8.5	15.2	9.0	4.6

2. 12.5 mM Borate buffer solutions, pH 9.3-10.7 at 25°C

Dissolve 0.476 g of Borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) in 70 ml of distilled water and add x ml of 0.1 N NaOH according to the pH required. Then

dilute to 100 ml.

pH	x ml
9.3	3.6
9.4	6.2
9.5	8.8
9.6	11.1
9.7	13.1
9.8	15.0
9.9	16.7
10.0	18.3

pH	x ml
10.1	19.5
10.2	20.5
10.3	21.3
10.4	22.1
10.5	22.7
10.6	23.3
10.7	23.8

3. 50 mM Glycine-HCl buffer solutions, pH 2.2-3.6 at 25°C

* Glycine, $C_2H_5NO_2$, M.W. 75.07

Dissolve 0.375 g of glycine in 70 ml of distilled water and add x ml of 0.2 N HCl according to the pH required. Then QS to 100 ml.

pH	x ml
2.2	22.0
2.4	16.2
2.6	12.1
2.8	8.4

pH	x ml
3.0	5.7
3.2	4.1
3.4	3.2
3.6	2.5

4. 50 mM Glycine-NaOH buffer solutions, pH 8.6-10.6 at 25°C

* Glycine, $C_2H_5NO_2$, M.W. 75.07

Dissolve 0.375 g of glycine in 70 ml of distilled water and add x ml of 0.2 N NaOH according to the pH required. Then QS to 100 ml.

pH	x ml
8.6	2.0
8.8	3.0
9.0	4.4
9.2	6.0
9.4	8.4

pH	x ml
9.6	11.2
9.8	13.6
10.0	16.0
10.4	19.3
10.6	22.75

5. 0.1 M Sodium carbonate-sodium bicarbonate buffer solutions, pH 9.2-10.5 at 20°C

* $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$, M.W. 286.2

* NaHCO_3 , M.W. 84.0

Dissolve x g of $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$ and y g of NaHCO_3 in distilled water according to the pH required and bring the total volume to 100 ml.

pH	x g	y g	pH	x g	y g
9.2	0.286	0.756	9.9	1.431	0.420
9.4	0.572	0.672	10.1	1.717	0.336
9.5	0.859	0.588	10.3	2.003	0.252
9.8	1.145	0.504	10.5	2.290	0.168

6. 25 mM Carbonate buffer solutions, pH 9.7-10.9 at 25°C

* NaHCO_3 , M.W. 84.0

Dissolve 0.21 g of NaHCO_3 in 70 ml of distilled water and add x ml of 0.1 M NaOH according to the pH required. Then dilute to 100 ml.

pH	x ml	pH	x ml
9.7	6.2	10.4	16.5
9.8	7.6	10.5	17.8
9.9	9.1	10.6	19.1
10.0	10.7	10.7	20.2
10.1	12.2	10.8	21.2
10.2	13.8	10.9	22.0
10.3	15.2		

7. 50 mM Succinic acid-NaOH buffer solutions, pH 3.8-6.0 at 25°C

* Succinic acid, $\text{C}_4\text{H}_6\text{O}_4$, M.W. 118.09

Dissolve 0.591 g of succinic acid in 50 ml of distilled water and add x ml of 0.2 N NaOH according to the pH required. Then QS to 100 ml.

pH	x ml	pH	x ml	pH	x ml	pH	x ml
3.8	7.5	4.4	16.7	5.0	26.7	5.6	37.5
4.0	10.0	4.6	20.0	5.2	30.3	5.8	40.7
4.2	13.3	4.8	23.5	5.4	34.2	6.0	43.5

8. Citric acid-Na₂HPO₄ (McIlvaine) buffer solutions, pH approx. 2.6-7.6

* Citric acid monohydrate, C₆H₈O₇ · H₂O, M.W. 210.14

* Na₂HPO₄ anhydrous, M.W. 141.98

* Na₂HPO₄ · 2H₂O, M.W. 178.05

Mix x ml of 0.1 M citric acid (21.01 g/l) and y ml of 0.2 M Na₂HPO₄ (Na₂HPO₄ anhydrous 28.4 g/l or Na₂HPO₄ · 2H₂O 35.61 g/l) according to the pH required.

pH	x ml	y ml	pH	x ml	y ml
2.6	89.10	10.90	5.2	46.40	53.60
2.8	84.15	15.85	5.4	44.25	55.75
3.0	79.45	20.55	5.6	42.00	58.00
3.2	75.30	24.70	5.8	39.55	60.45
3.4	71.50	28.50	6.0	36.85	63.15
3.6	67.80	32.20	6.2	33.90	66.10
3.8	64.50	35.50	6.4	30.75	69.25
4.0	61.45	38.55	6.6	27.25	72.75
4.2	58.60	41.40	6.8	22.75	77.25
4.4	55.90	44.10	7.0	17.65	82.35
4.6	53.25	46.75	7.2	13.05	86.95
4.8	50.70	49.30	7.4	9.15	90.85
5.0	48.50	51.50	7.6	6.35	93.65

9. 0.1 M Citric acid-sodium citrate buffer solutions, pH 3.0-6.2

* Citric acid monohydrate, C₆H₈O₇ · H₂O, M.W. 210.14

* Trisodium citrate dihydrate, C₆H₅O₇Na₃ · 2H₂O, M.W. 294.12

Dissolve x g of citric acid and y g of trisodium citrate in distilled water according to the pH required and make up to 100 ml.

pH	x g	y g	pH	x g	y g
3.0	1.723	0.529	4.8	0.840	1.765
3.2	1.628	0.662	5.0	0.735	1.912
3.4	1.534	0.794	5.2	0.641	2.044
3.6	1.439	0.926	5.4	0.536	2.191
3.8	1.334	1.073	5.6	0.441	2.323
4.0	1.240	1.206	5.8	0.336	2.470
4.2	1.135	1.353	6.0	0.242	2.603
4.4	1.040	1.485	6.2	0.168	2.706
4.6	0.935	1.632			

10. 0.2 M Sodium acetate-acetic acid buffer solutions, pH approx. 3.7-5.6

* Sodium acetate trihydrate, $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$, M.W. 136.09

* Acetic acid glacial, CH_3COOH , M.W. 60.05

Mix x g of sodium acetate and y ml of glacial acetic acid according to the required pH and make up to 100 ml.

pH	x g	y ml	pH	x g	y ml
3.7	0.272	1.081	4.8	1.606	0.492
3.8	0.327	1.057	5.0	1.905	0.360
4.0	0.490	0.985	5.2	2.150	0.252
4.2	0.721	0.883	5.4	2.341	0.168
4.4	1.007	0.757	5.6	2.477	0.108
4.6	1.334	0.613			

11. 0.1 M Na_2HPO_4 - NaH_2PO_4 buffer solutions, pH 5.8-8.0 at 25°C

* Na_2HPO_4 anhydrous, M.W. 141.96; 0.2 M solution contains 28.39 g/l

* $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, M.W. 178.05; 0.2 M solution contains 35.61 g/l

* $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, M.W. 358.22; 0.2 M solution contains 71.64 g/l

* $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, M.W. 138.01; 0.2 M solution contains 27.60 g/l

* $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, M.W. 156.03; 0.2 M solution contains 31.21 g/l

Mix x ml of 0.2 M Na_2HPO_4 and y ml of 0.2 M NaH_2PO_4 and dilute to 100 ml with distilled water.

pH	x ml	y ml
5.8	4.0	46.0
6.0	6.15	43.85
6.2	9.25	40.75
6.4	13.25	36.75
6.6	18.75	31.25
6.8	24.5	25.5

pH	x ml	y ml
7.0	30.5	19.5
7.2	36.0	14.0
7.4	40.5	9.5
7.6	43.5	6.5
7.8	45.75	4.25
8.0	47.35	2.65

12. 50 mM Triethanolamine hydrochloride-NaOH buffer solutions, pH 6.8-8.6 at 20°C

* Triethanolamine hydrochloride, $C_6H_{15}NO_3 \cdot HCl$, M.W. 185.7

The hydrochloride is prepared by dissolving pure triethanolamine in 1 volume of ethanol, neutralising with conc. HCl, and cooling to 0°C. Dissolve 18.57 g of triethanolamine hydrochloride in 50 ml of distilled water and add x ml of 0.1 N NaOH according to the pH required. Then Make up to 100 ml with distilled water.

pH	x ml
6.8	6.0
7.0	9.0
7.2	12.15
7.4	15.8
7.6	20.05

pH	x ml
7.8	26.0
8.0	31.5
8.2	36.0
8.4	40.0
8.6	46.0

13. 50 mM Tris (hydroxymethyl) aminomethane-HCl buffer solutions, pH 7.1-8.9 at 25°C

* Tris, $C_4H_{11}NO_3$, M.W. 121.14

Dissolve 0.606 g of tris in 50 ml of distilled water and add x ml of 0.1 M HCl according to the required pH. Dilute to 100 ml with H_2O .

pH	x ml	pH	x ml
7.1	45.7	8.1	26.2
7.2	44.7	8.2	22.9
7.3	43.4	8.3	19.9
7.4	42.0	8.4	17.2
7.5	40.3	8.5	14.7
7.6	38.5	8.6	12.4
7.7	36.6	8.7	10.3
7.8	34.5	8.8	8.5
7.9	32.0	8.9	7.0
8.0	29.2		

NOTE: Tris solutions absorb CO₂ from the air.

0.1 N HCl

Dilute conc. HCl (commercial, 38%) 116 times with distilled water (8.621 ml/l).

0.1 N NaOH

Dissolve 4 g of NaOH in 1 litre of distilled water.

PBS (10X)

Na ₂ HPO ₄ anhydrous	11.5 g
(Na ₂ HPO ₄ • 2H ₂ O	14.42 g)
(Na ₂ HPO ₄ • 12H ₂ O	29.01 g)
KH ₂ PO ₄	2 g
KCl	2 g
NaCl	80 g
Distilled Water	1 litre