

Preparation of Buffers for Use in Enzyme Studies (by G. Gomori)

The buffers described in this section are suitable for use either in enzymatic or histochemical studies. The accuracy of the tables is within ± 0.05 pH at 23 °C. In most cases the pH values will not be off by more than ± 0.02 pH even at 37 °C and at molarities slightly different from those given (usually 0.05 M).

The methods of preparation described are not necessarily identical with those of the original authors. The titration curves of the majority of the buffers recommended have been redetermined by the writer. The buffers are arranged in the order of ascending pH range.

1. Hydrochloric Acid-Potassium Chloride Buffer.

Stocks solutions

A: 0.2 M solution of KCl (14.91 g in 1 L)

B: 0.2 M HCl

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH
97.0	1.0
78.0	1.1
64.5	1.2
51.0	1.3
41.5	1.4
33.3	1.5
26.3	1.6
20.6	1.7
16.6	1.8
13.2	1.9
10.6	2.0
8.4	2.1
6.7	2.2

2. Glycine-HCl Buffer.

Stocks solutions

A: 0.2 M solution of glycine (15.01 g in 1 L)

B: 0.2 M HCl

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
5.0	3.6	16.8	2.8
6.4	3.4	24.2	2.6
8.2	3.2	32.4	2.4
11.4	3.0	44.0	2.2

3. Phthalate-Hydrochloric Acid Buffer.

Stocks solutions

A: 0.2 M solution of potassium acid phthalate (40.48 g in 1 L)

B: 0.2 M HCl

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
46.7	2.2	14.7	3.2
39.6	2.4	9.9	3.4
33.0	2.6	6.0	3.6
26.4	2.8	2.63	3.8
20.3	3.0		

4. Aconitate Buffer.

Stocks solutions

A: 0.5 M solution of aconitic acid (87.05 g in 1 L)

B: 0.2 M NaOH

20 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
15.0	2.5	83.0	4.3
21.0	2.7	90.0	4.5
28.0	2.9	97.0	4.7
36.0	3.1	103.0	4.9
44.0	3.3	108.0	5.1
52.0	3.5	113.0	5.3
60.0	3.7	119.0	5.5
68.0	3.9	126.0	5.7
76.0	4.1		

5. Citrate Buffer.

Stocks solutions

A: 0.1 M solution of citric acid (21.01 g in 1 L)

B: 0.1 M solution of sodium citrate (29.41 g $C_6H_5O_7Na_3 \cdot 2H_2O$ in 1 L, the use of the salt with $5\frac{1}{2} H_2O$ is not recommended).

x mL of A + y mL of B, diluted to a total of 100 mL

x	y	pH
46.5	3.5	3.0
43.7	6.3	3.2
40.0	10.0	3.4
37.0	13.0	3.6
35.0	15.0	3.8
33.0	17.0	4.0
31.5	18.5	4.2
28.0	22.0	4.4
25.5	24.5	4.6
23.0	27.0	4.8
20.5	29.5	5.0
18.0	32.0	5.2
16.0	34.0	5.4
13.7	36.3	5.6
11.8	38.2	5.8
9.5	41.5	6.0
7.2	42.8	6.2

6. Acetate Buffer.

Stocks solutions

A: 0.2 M solution of acetic acid (11.55 g in 1 L)

B: 0.2 M solution of sodium acetate (16.4 g $C_2H_3O_2Na$ or 27.2 g of $C_2H_3O_2Na \cdot 3H_2O$ in 1 L).

x mL of A + y mL of B, diluted to a total of 100 mL

x	y	pH
46.3	3.7	3.6
44.0	6.0	3.8
41.0	9.0	4.0
36.8	13.2	4.2
30.5	19.5	4.4
25.5	24.5	4.6
20.0	30.0	4.8
14.8	35.2	5.0
10.5	39.5	5.2
8.8	41.2	5.4
4.8	45.2	5.6

7. Citrate-Phosphate Buffer.

Stocks solutions

A: 0.1 M solution of citric acid (19.21 g in 1 L)

B: 0.2 M solution of dibasic sodium phosphate (53.65 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ or 71.7 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in 1 L).

x mL of A + y mL of B, diluted to a total of 100 mL

x	y	pH
44.6	5.4	2.6
42.2	7.8	2.8
39.8	10.2	3.0
37.7	12.3	3.2
35.9	14.1	3.4
33.9	16.1	3.6
32.3	17.7	3.8
30.7	19.3	4.0
29.4	20.6	4.2
27.8	22.2	4.4
26.7	23.3	4.6
25.2	24.8	4.8
24.3	25.7	5.0
23.3	26.7	5.2
22.2	27.8	5.4
21.0	29.0	5.6
19.7	30.3	5.8
17.9	32.1	6.0
16.9	33.1	6.2
15.4	34.6	6.4
13.6	36.4	6.6
9.1	40.9	6.8
6.5	43.6	7.0

8. Succinate Buffer.

Stocks solutions

A: 0.2 M solution of succinic acid (23.6 g in 1L)

B: 0.2 M NaOH

25 mL of A + x mL of B, diluted to a total of 100 mL

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

9. Phthalate-Sodium Hydroxide Buffer.

Stocks solutions

A: 0.2 M solution of potassium acid phthalate (40.84 g in 100 mL)

B: 0.2 M NaOH

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
3.7	4.2	30.0	5.2
7.5	4.4	35.5	5.4
12.2	4.6	39.8	5.6
17.7	4.8	43.0	5.8
23.9	5.0	45.5	6.0

10. Maleate Buffer.

Stocks solutions

A: 0.2 M solution of acid sodium maleate (8 g of NaOH + 23.2 g of maleic acid or 19.6 g of maleic anhydride in 1 L)

B: 0.2 M NaOH

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
7.2	5.2	33.0	6.2
10.5	5.4	38.0	6.4
15.3	5.6	41.6	6.6
20.8	5.8	44.4	6.8
26.9	6.0		

11. Cacodylate Buffer.

Stocks solutions

A: 0.2 M solution of sodium cacodylate (42.8 g of $\text{Na}(\text{CH}_3)_2\text{AsO}_2 \cdot 3\text{H}_2\text{O}$ in 1 L)

B: 0.2 M HCl

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
2.7	7.4	29.6	6.0
4.2	7.2	34.8	5.8
6.3	7.0	39.2	5.6
9.3	6.8	43.0	5.4
13.3	6.6	45.0	5.2
18.3	6.4	47.0	5.0
23.8	6.2		

12. Phosphate Buffer.

Stocks solutions

A: 0.2 M solution of monobasic sodium phosphate (27.8 g in 1 L)

B: 0.2 M solution of dibasic sodium phosphate (53.65 g of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ or 71.7 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in 1 L).

x mL of A + y mL of B, diluted to a total of 200 mL

x	y	pH	x	y	pH
93.5	6.5	5.7	45.0	55.0	6.9
92.0	8.0	5.8	39.0	61.0	7.0
90.0	10.0	5.9	33.0	67.0	7.1
87.7	12.3	6.0	28.0	72.0	7.2
85.0	15.0	6.1	23.0	77.0	7.3
81.5	18.5	6.2	19.0	81.0	7.4
77.5	22.5	6.3	16.0	84.0	7.5
73.5	26.5	6.4	13.0	87.0	7.6
68.5	31.5	6.5	10.5	90.5	7.7
62.5	37.5	6.6	8.5	91.5	7.8
56.5	43.5	6.7	7.0	93.0	7.9
51.0	49.0	6.8	5.3	94.7	8.0

13. Tris(hydroxymethyl) aminomethane-maleate (Tris-maleate) Buffer.

Stocks solutions

A: 0.2 M solution of Tris acid maleate (24.2 g of tris(hydroxymethyl) aminomethane + 23.2 g of maleic acid or 19.6 g of maleic anhydride in 1 L)

B: 0.2 M NaOH

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
7.0	5.2	48.0	7.0
10.8	5.4	51.0	7.2
15.5	5.6	54.0	7.4
20.5	5.8	58.0	7.6
26.0	6.0	63.5	7.8
31.5	6.2	69.0	8.0
37.0	6.4	75.0	8.2
42.5	6.6	81.0	8.4
45.0	6.8	86.5	8.6

14. Barbitol Buffer.

Stocks solutions

A: 0.2 M solution of sodium barbitol (veronal) (41.2 g in 1 L)

B: 0.2 M HCl

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH
1.5	9.2
2.5	9.0
4.0	8.8
6.0	8.6
9.0	8.4
12.7	8.2
17.5	8.0
22.5	7.8
27.5	7.6
32.5	7.4
39.0	7.2
43.0	7.0
45.0	6.8

Solutions more concentrated than 0.05 M may crystallize on standing, especially in the cold.

15. Tris(hydroxymethyl) aminomethane (Tris) Buffer.

Stocks solutions

A: 0.2 M solution of tris(hydroxymethyl) aminomethane (24.2 g in 1 L)

B: 0.2 M HCl

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH
5.0	9.0
8.1	8.8
12.2	8.6
16.5	8.4
21.9	8.2
26.8	8.0
32.5	7.8
38.4	7.6
41.4	7.4
44.2	7.2

16. Boric Acid-Borax Buffer.

Stocks solutions

A: 0.2 M solution of boric acid (12.4 g in 1 L)

B: 0.05 M solution of borax (19.05 g in 1 L; 0.2 M in terms of sodium borate)

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
2.0	7.6	22.5	8.7
3.1	7.8	30.0	8.8
4.9	8.0	42.5	8.9
7.3	8.2	59.0	9.0
11.5	8.4	83.0	9.1
17.5	8.6	115.0	9.2

17. 2-amino-2methyl-1,3-propanediol (Ammediol) Buffer.

Stocks solutions

A: 0.2 M solution of 2-amino-2-methyl-1,3-propanediol (21.03 g in 1 L)

B: 0.2 M HCl

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
2.0	10.0	22.0	8.8
3.7	9.8	29.5	8.6
5.7	9.6	34.0	8.4
8.5	9.4	37.7	8.2
12.5	9.2	41.0	8.0
16.7	9.0	43.5	7.8

18. Glycine-NaOH Buffer.

Stocks solutions

A: 0.2 M solution of glycine (15.01 g in 1 L)

B: 0.2 M NaOH

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH	x	pH
4.0	8.6	22.4	9.6
6.0	8.8	27.2	9.8
8.8	9.0	32.0	10.0
12.0	9.2	38.6	10.4
16.8	9.4	45.5	10.6

19. Borax-NaOH Buffer.

Stocks solutions

A: 0.05 M solution of borax (19.05 g in 1 L; 0.02 M in terms of sodium borate)

B: 0.2 M NaOH

50 mL of A + x mL of B, diluted to a total of 200 mL

x	pH
0.0	9.28
7.0	9.35
11.0	9.4
17.6	9.5
23.0	9.6
29.0	9.7
34.0	9.8
38.6	9.9
43.0	10.0
46.0	10.1

5. Carbonate-Bicarbonate Buffer.

Stocks solutions

A: 0.2 M solution of anhydrous sodium carbonate (21.2 g in 1 L)

B: 0.2 M solution of sodium bicarbonate (16.8 g in 1 L)

x mL of A + y mL of B, diluted to a total of 200 mL

x	y	pH
4.0	46.0	9.2
7.5	42.5	9.3
9.5	40.5	9.4
13.0	37.0	9.5
16.0	34.0	9.6
19.5	30.5	9.7
22.0	28.0	9.8
25.0	25.0	9.9
27.5	22.5	10.0
30.0	20.0	10.1
33.0	17.0	10.2
35.5	14.5	10.3
38.5	11.5	10.4
40.5	9.5	10.5
42.5	7.5	10.6
45.0	5.0	10.7