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Preparation of buffers
(Methods in Enzimology, Vol. 1, pag 138)

1. HYDROCHLORIC ACID-POTASSIUM CHLORIDE BU-
FFER (1)

Stock Solutions

a: 0.2 M solution of KCl (14.91 g. in 1000 ml.).

b: 0.2 M HCl
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

<u>X</u>	<u>pH</u>
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

(1) W.M.Clark & H.A.Lubs, J.Bacteriol.
(1917).

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2. GLYCINE-HCl BUFFER (2)

Stock Solutions

A: 0.2 M solution of glycine (15.01 g. in 1000 ml.).

B: 0.2 M HCl

50 ml. of A + x ml. of b, diluted a total of 200 ml.

<u>x</u>	<u>pH</u>	<u>x</u>	<u>pH</u>
5.0	3.6	16.8	2.8
6.4	2.4	24.2	2.6
8.2	3.2	32.4	2.4
11.4	3.0	44.0	2.2

(2) S.P.L. Sorensen, Biochem. Z 2, 131. (1909
22, 352 (1909)).

3. PHTHALATE-HYDROCHLORIC ACID BUFFER (1)

Stock solutions

A: 0.2 M solution of potassium acid ohthalate
(40.84 g. in 1000 ml.).

B: 0.2 M CHL

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

<u>x</u>	<u>pH</u>	<u>X</u>	<u>pH</u>
46,7	2,2	14,7	3,2
39,6	2,4	9,9	3,4
33,0	2,6	6,0	3,4
26,4	2,8	2,63	3,8
20,3	3,0		

(1) W.M.Clark & H.A:Lubs. J.Bacteriol. 21.
(1917)

5. CITRATE BUFFER (4)

Stock solutions

A: 0.1 M solution of citric acid (21.01 g. in 1000 ml.

B: 0.1 M solution of sodium citrate (29.41 g. $\text{C}_6\text{H}_5\text{O}_7\text{N}_3 \cdot 2\text{H}_2\text{O}$ in 1000 ml.: the use of the salt with $5\frac{1}{2}\text{H}_2\text{O}$ is not recommended).

x ml. of A -! ml. of B, diluted to a total of 100 ml.

<u>x</u>	<u>Y</u>	<u>pH</u>
46,5	3,5	3,0
43,7	6,3	3,2
40,0	10,0	4,3
37,0	15,0	4,6
35,0	15,0	3,8
33,0	17,0	4,0
31,5	18,5	4,2
28,0	22,0	5,4
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
8,5	41,5	6,0
7,2	42,8	6,2

(4) R.D. Lillie. "Histopathologic Technique, Blakiston". Philadelphia & Toronto, 1948).

6. ACETATE BUFFER (5)

Stock solutions

A: 0.2M solution of acetic acid(11.95)ml. in 1000 ml.).

B: 0.2M solution of sodium acetate(16.4 g.of $\text{C}_2\text{H}_3\text{O}_2\text{Na}$ or 27,2 of $\text{C}_2\text{H}_3\text{O}_2\text{Na}\cdot 3\text{H}_2\text{O}$ in 1000 ml.).

x ml. of A + y ml. of B, doluted to a total of 100 ml.

<u>x</u>	<u>Y</u>	<u>pH</u>
46,3	3,7	3,6
44,0	6,0	3,8
41,0	9,0	4,0
36,9	13,2	4,3
30,5	18,5	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	45,3	5,6

(5) G.S.Walpol's, J.Chem.Soc.105,2501 (1914).

12. PHOSPHATE BUFFER (2)

Stock solutions

A: 0.2 M solution of monobasic sodium phosphate
27.8 g. in 1000 ml. NaH_2PO_4

B: 0.2 M solution of dibasic sodium phosphate
(53.65 g. of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ or 71.7 g. of $\text{Na}_2\text{HOP}_4 \cdot 12\text{H}_2\text{O}$)

x ml. of A + y ml. of b, diluted to a total of 200 ml.

<u>X</u>	<u>Y</u>	<u>pH</u>	<u>X</u>	<u>Y</u>	<u>pH</u>
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	14,0	13,0	7,6
68,5	31,5	6,5	10,5	9,5	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

(2) S.P.L. Sorensen. Biochem. Z. 21, 131 (1909), 22, 352 (1909).

7. CITRATE-PHOSPHATE BUFFER (6)

Stock solutions

X A: 0.1 M solution of citric acid (19.21 g. in 1000 ml.).

Y B: 0.2 M solution of dibasic sodium phosphate (53.65 of Na_2HPO_4 / of 71.7 g. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in 100 ml.

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	12,4	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

(6) T.C. Mellwaine, J. Biol. Chem. 49, 183 (1921)

8. SUCCINATE BUFFER (3)

Stock solutions

A: 0.2 M solution of succinic acid (23.6 g. in 1000 ml.).

B: 0.2 M NaCH

25 ml. of A x ml. of B. diluted to a total of 100 ml.

<u>X</u>	<u>pH</u>	<u>X</u>	<u>pH7</u>
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	4,8
23,5	4,8	43,5	6,0

(3) G. Gomori. Unpublished.

9. PHTHALATE-SODIUM HYDROXINE BUFFER (1)

Stock solutions

A: 0.2M 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.

<u>X</u>	<u>pH</u>	<u>X</u>	<u>pH</u>
3,7	4,2	30,0	5,2
7,5	4,4	35,5	5,4
12,2	4,4	39,8	5,6
17,7	4,8	43,0	5,8
23,9	5,0	45,5	6,0

(1) W.M.Clark & H.A. Lubs, J. Bacteriol. 21,1
(1917).

10. MALEATE BUFFER (7)

Stock solutions

A: 0.2M solution of acid sodium maleate (8. g. of NaOH + 23.2 of maleic acid or 19.6 g. of maleic anhydride in 1000 ml.)

B: 0.2 M NaOH

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

<u>X</u>	<u>pH</u>	<u>X</u>	<u>pH</u>
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	5,8
26,9	6,0		

(7) J.W. Temple. J. Am. Chem. Soc. 51, 1754 (1929)

20. CARBONATE-BICARBONATE BUFFER (14)

Stock solutions

A: 0.2M solution of anhydrous sodium carbonate (21.2 g. in 1000 ml.)

B: 0.2 M solution of sodium bicarbonate (18.8 g in 1000 ml.) x ml. of A + of B, diluted to a total of 200.

<u>X</u>	<u>Y</u>	<u>pH</u>
4,0	46,0	0,2
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	6,0	10,7

(14) G.E. Delory & E.J. King-Biochem.J.19.245(1945)