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Buffers for Use in Biological Systems

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We use this protocol and it's working

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Abstract

A buffer solution has the function of resisting changes in pH even when adding powerful acids or bases. However, in the physiological environment the buffered system also provides cofactors for enzymatic reactions, critical salts and even essential nutrients for cells and tissues. Therefore, when trying to reproduce biological conditions *in vitro*, we must make the appropriate choice of the buffer. After all, it will provide the appropriate medium in which reactions will occur.

Safety warnings

Wear personal protective equipment: gloves, lab coat and mask.

Before start

Organize your workspace.

Make sure all solutions and equipment are available. Plan the experiment!

Acetate Buffer

1 pH range: 3.6 to 5.6

(a) 0.1 M Acetic acid (5.8 mL made to 1000 mL)
(b) 0.1 M Sodium acetate; 8.2 g L^{-1} (anhydrous; M.W. 82.0 g mol^{-1}) or 13.6 g L^{-1} (trihydrate; M.W. 136.0 g mol^{-1})

Mix acetic acid and sodium acetate solutions in the proportions indicated
Adjust the final volume to 100 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of Acetic acid	46.3	41.0	30.5	20.0	14.8	10.5	4.8
mL of Sodium acetate	3.7	9.0	19.5	30.0	35.2	39.5	45.2
pH	3.6	4.0	4.4	4.8	5.0	5.2	5.6

Glycine-HCl Buffer

2 pH range: 2.2 to 3.6

(a) 0.1 M Glycine: 7.5 g L^{-1} (M.W.: 75.0 g mol^{-1})
(b) 0.1 M Hydrochloric acid

Mix 50 mL of glycine and indicated volume of hydrochloric acid
Mix and adjust the final volume to 100 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of HCl	44.0	32.4	24.2	16.8	11.4	8.2	6.4	3.6
pH	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6

Hydrochloric Acid-Potassium Chloride Buffer (HCl-KCl)

3 pH Range: 1.0 to 2.2

(a) 0.1 M Potassium chloride: 7.45 g L^{-1} (M.W.: 74.5 g mol^{-1})
(b) 0.1 M Hydrochloric acid

Mix 50 mL of potassium chloride and indicated volume of hydrochloric acid
Mix and adjust the final volume to 100 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of HCl	97	64.5	41.5	26.3	16.6	10.6	6.7
pH	1.0	1.2	1.4	1.6	1.8	2.0	2.2

Citrate-Phosphate Buffer

4 pH range: 2.6 to 7.0

(a) 0.1 M Citric acid; 19.21 g L^{-1} (M.W. 192.1 g mol^{-1})
(b) 0.2 M Dibasic sodium phosphate; 35.6 g L^{-1} (dihydrate; M.W. 178.0 g mol^{-1}) or 53.6 g L^{-1} (heptahydrate; M.W. 268.0 g mol^{-1})

Mix citric acid and sodium phosphate solutions in the proportions indicated and adjust the final volume to 100 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of Citric acid	44.6	39.8	35.9	32.3	29.4	26.7	24.3	22.2	19.7	16.9	13.6	6.5
mL of Sodium phosphate	5.4	10.2	14.1	17.7	20.6	23.3	25.7	27.8	30.3	33.1	36.4	43.6
pH	2.6	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2	6.6	7.0

Citrate Buffer

5 pH range: 3.0 to 6.2

(a) 0.1 M Citric acid: 19.21 g L^{-1} (M.W.: 192.1 g mol^{-1})
(b) 0.1 M Sodium citrate dihydrate: 29.4 g/l (M.W.: 294.0 g mol^{-1})

Mix citric acid and sodium citrate solutions in the proportions indicated and adjust the final volume to 100 mL with deionized water
Adjust the final pH using a sensitive pH meter
The use of pentahydrate salt of sodium citrate is not recommended

mL of Citric acid	46.5	40.0	35.0	31.5	25.5	20.5	16.0	11.8	7.2
mL of Sodium citrate	3.5	10	15.0	18.5	24.5	29.5	34.0	38.2	42.8
pH	3.0	3.4	3.8	4.2	4.6	5.0	5.4	5.8	6.2

Phosphate Buffer

6 pH range: 5.8 to 8.0

(a) 0.1 M Sodium phosphate monobasic; 13.8 g L^{-1} (monohydrate, M.W. 138.0 g mol^{-1})
(b) 0.1 M Sodium phosphate dibasic; 26.8 g L^{-1} (heptahydrate, M.W. 268.0 g mol^{-1})

Mix sodium phosphate monobasic and dibasic solutions in the proportions indicated
Adjust the final volume to 200 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of Sodium phosphate, Monobasic	92.0	81.5	73.5	62.5	51.0	39.0	28.0	19.0	13.0	8.5	5.3
mL of Sodium phosphate, Dibasic	8.0	18.5	26.5	37.5	49.0	61.0	72.0	81.0	87.0	91.5	94.7
pH	5.8	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0

Tris-HCl Buffer

7 pH range: 7.2 to 9.0

(a) 0.1 M Tris(hydroxymethyl)aminomethane; 12.1 g L^{-1} (M.W.: 121.0 g mol^{-1})
(b) 0.1 M Hydrochloric acid

Mix 50 mL of Tris(hydroxymethyl)aminomethane and indicated volume of hydrochloric acid

Adjust the final volume to 200 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of HCl	44.2	41 .4	38.4	32.5	21 .9	12.2	5.0
pH	7.2	7.4	7.6	7.8	8.2	8.6	9.0

Carbonate-Bicarbonate Buffer

8 pH range: 9.2 to 10.6

(a) 0.1 M Sodium carbonate (anhydrous), 10.6 g L^{-1} (M.W.: 106.0 g mol^{-1})
(b) 0.1 M Sodium bicarbonate, 8.4 g L^{-1} (M.W.: 84.0 g mol^{-1})

Mix sodium carbonate and sodium bicarbonate solutions in the proportions indicated
Adjust the final volume to 200 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of Sodium carbonate	4.0	9.5	16.0	22.0	27.5	33.0	38.5	42.5
mL of Sodium bicarbonate	46.0	40.5	34.0	28.0	22.5	17.0	11.5	7.5
pH	9.2	9.4	9.6	9.8	10.0	10.2	10.4	10.6

Glycine-Sodium Hydroxide Buffer

9 pH: 8.6 to 10.6

(a) 0.1 M Glycine; 7.5 g L^{-1} (M.W.: 75.0 g mol^{-1})
(b) 0.1 M Sodium hydroxide; 4.0 g L^{-1} (M.W.: 40.0 g mol^{-1})

Mix 50 mL of glycine and indicated volume of sodium hydroxide solutions
Adjust the final volume to 200 mL with deionized water
Adjust the final pH using a sensitive pH meter

mL of Sodium hydroxide	4.0	8.8	16.8	27.2	32.0	38.6	45.5

	pH	8.6	9.0	9.4	9.8	10.0	10.4	10.6

Tris Buffred Saline (TBS)

10 pH 7.4

10 mM Tris
150 mM NaCl

1 L of TBS can be prepared by dissolving 1.21 g of Tris base and 8.7 g of NaCl in 1 L of distilled water
Adjust the pH before use

Note: Tris has a pK_a of 8.3. Hence, the buffering capacity at pH 7.4 is minimal compared to phosphate buffer (pK_a = 7.21)

Phosphate Buffered Saline (PBS)

11 pH 7.4

150 mM NaCl
10 mM Potassium Phosphate buffer

1 L PBS can be prepared by dissolving 8.7 g NaCl, 1.82 g $K_2HPO_4 \bullet 3H_2O$, and 0.23 g KH_2PO_4 in 1 L of distilled water
Adjust the pH before use

A variation of PBS can also be prepared as follows:
137 mM NaCl
2.7 mM KCl
10 mM Na_2HPO_4
1.76 mM KH_2PO_4