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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.

## Troubleshooting

## 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<sup>-1</sup> (anhydrous; M.W. 82.0 g mol<sup>-1</sup>) or 13.6 g L<sup>-1</sup> (trihydrate; M.W. 136.0 g mol<sup>-1</sup>)

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

<b>mL of Acetic acid</b>	46.3	41.0	30.5	20.0	14.8	10.5	4.8
<b>mL of Sodium acetate</b>	3.7	9.0	19.5	30.0	35.2	39.5	45.2
<b>pH</b>	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<sup>-1</sup> (M.W.: 75.0 g mol<sup>-1</sup>)

(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

<b>mL of HCl</b>	44.0	32.4	24.2	16.8	11.4	8.2	6.4	3.6
<b>pH</b>	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<sup>-1</sup> (M.W.: 74.5 g mol<sup>-1</sup>)

(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

<b>mL of HCl</b>	97	64.5	41.5	26.3	16.6	10.6	6.7
<b>pH</b>	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<sup>-1</sup> (M.W. 192.1 g mol<sup>-1</sup>)

(b) 0.2 M Dibasic sodium phosphate; 35.6 g L<sup>-1</sup> (dihydrate; M.W. 178.0 g mol<sup>-1</sup>) or 53.6 g L<sup>-1</sup> (heptahydrate; M.W. 268.0 g mol<sup>-1</sup>)

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

<b>mL of Citric acid</b>	44.6	39.8	35.9	32.3	29.4	26.7	24.3	22.2	19.7	16.9	13.6	6.5
<b>mL of Sodium phosphate</b>	5.4	10.2	14.1	17.7	20.6	23.3	25.7	27.8	30.3	33.1	36.4	43.6
<b>pH</b>	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<sup>-1</sup> (M.W.: 192.1 g mol<sup>-1</sup>)

(b) 0.1 M Sodium citrate dihydrate: 29.4 g/l (M.W.: 294.0 g mol<sup>-1</sup>)

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

<b>mL of Citric acid</b>	46.5	40.0	35.0	31.5	25.5	20.5	16.0	11.8	7.2
<b>mL of Sodium citrate</b>	3.5	10	15.0	18.5	24.5	29.5	34.0	38.2	42.8
<b>pH</b>	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<sup>-1</sup> (monohydrate, M.W. 138.0 g mol<sup>-1</sup>)

(b) 0.1 M Sodium phosphate dibasic; 26.8 g L<sup>-1</sup> (heptahydrate, M.W. 268.0 g mol<sup>-1</sup>)

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

<b>mL of Sodium phosphate, Monobasic</b>	92.0	81.5	73.5	62.5	51.0	39.0	28.0	19.0	13.0	8.5	5.3
<b>mL of Sodium phosphate, Dibasic</b>	8.0	18.5	26.5	37.5	49.0	61.0	72.0	81.0	87.0	91.5	94.7
<b>pH</b>	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<sup>-1</sup> (M.W.: 121.0 g mol<sup>-1</sup>)

(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

<b>mL of HCl</b>	44.2	41.4	38.4	32.5	21.9	12.2	5.0
<b>pH</b>	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<sup>-1</sup> (M.W.: 106.0 g mol<sup>-1</sup>)

(b) 0.1 M Sodium bicarbonate, 8.4 g L<sup>-1</sup> (M.W.: 84.0 g mol<sup>-1</sup>)

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

<b>mL of Sodium carbonate</b>	4.0	9.5	16.0	22.0	27.5	33.0	38.5	42.5
<b>mL of Sodium bicarbonate</b>	46.0	40.5	34.0	28.0	22.5	17.0	11.5	7.5
<b>pH</b>	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<sup>-1</sup> (M.W.: 75.0 g mol<sup>-1</sup>)  
 (b) 0.1 M Sodium hydroxide; 4.0 g L<sup>-1</sup> (M.W.: 40.0 g mol<sup>-1</sup>)

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

	<b>mL of Sodium hydroxide</b>	4.0	8.8	16.8	27.2	32.0	38.6	45.5
	<b>pH</b>	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<sub>a</sub> of 8.3. Hence, the buffering capacity at pH 7.4 is minimal compared to phosphate buffer (pK<sub>a</sub> = 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<sub>2</sub>HPO<sub>4</sub> • 3H<sub>2</sub>O, and 0.23 g KH<sub>2</sub>PO<sub>4</sub> in 1 L of distilled water  
 Adjust the pH before use

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A variation of PBS can also be prepared as follows:

137 mM NaCl  
2.7 mM KCl  
10 mM Na<sub>2</sub>HPO<sub>4</sub>  
1.76 mM KH<sub>2</sub>PO<sub>4</sub>