BUFFERS

INTRODUCTION

Presented are tables to assist with the selection and preparation of commonly used buffers for use in biotechnology laboratories.

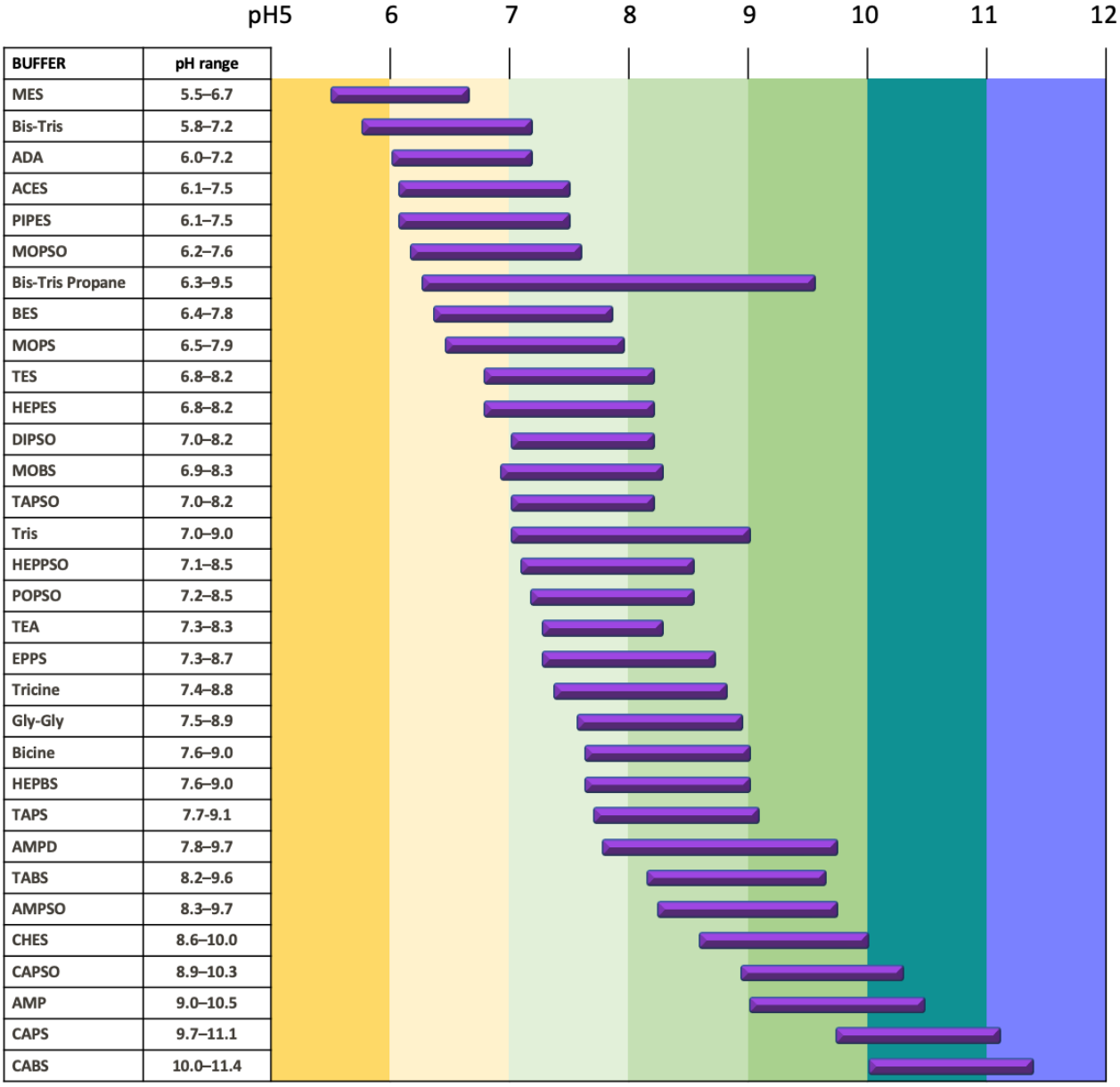
- Biological buffers
- Blended buffers
- Other common buffer recipes

BIOLOGICAL BUFFERS

The control and maintenance of pH in biological experiments is achieved by selection of a suitable buffering agent. The selection of which buffer to use should be considered carefully, taking into account factors such as what concentration of buffer reagent is required to give sufficient buffering capacity? What is the target pH and does that target sit comfortably within the pH range of the buffer? (See Figure 1). Most of the commonly used biological buffers are based upon those originally proposed by Good *et al* [1], [2]. A summary of the useful pH ranges of these buffers is given in Figure 1 and Table 1 below. Ideally the target pH for the experiment should be near the middle of the pH range for the buffer that is chosen.

There are other considerations that should be taken into account. Such as, are there likely to be temperature changes during the experiment? And if so, is the buffer reagents pKa affected by temperature? (Tris notoriously changes pH as temperature changes). Is the cost of the buffer reagent a factor? What is the volume of acid or base that you will need to add to adjust the pH and is that a factor for consideration? Does the buffer have properties that may cause undesired affects that will impact the experiment? For example, does it chelate metal ions? Ferreira *et al* [3] reviews criteria to consider when selecting a buffer system.

Figure 1: Useful pH ranges of common biological buffers



BUFFER	pH range
MES	5.5–6.7
Bis-Tris	5.8–7.2
ADA	6.0–7.2
ACES	6.1–7.5
PIPES	6.1–7.5
MOPSO	6.2–7.6
Bis-Tris Propane	6.3–9.5
BES	6.4–7.8
MOPS	6.5–7.9
TES	6.8–8.2
HEPES	6.8–8.2
DIPSO	7.0–8.2
MOBS	6.9–8.3
TAPSO	7.0–8.2
Tris	7.0–9.0
HEPPSO	7.1–8.5
POPSO	7.2–8.5
TEA	7.3–8.3
EPPS	7.3–8.7
Tricine	7.4–8.8
Gly-Gly	7.5–8.9
Bicine	7.6–9.0
HEPBS	7.6–9.0
TAPS	7.7-9.1
AMPD	7.8–9.7
TABS	8.2–9.6
AMPSO	8.3–9.7
CHES	8.6–10.0
CAPSO	8.9–10.3
AMP	9.0–10.5
CAPS	9.7–11.1
CABS	10.0–11.4

Table 1 : Useful pH ranges and pka of common biological buffers

Buffers	Useful pH Range	pKa (at 25°C)
MES	5.5–6.7	6.10
Bis-Tris	5.8–7.2	6.50
ADA	6.0–7.2	6.59
ACES	6.1–7.5	6.78
PIPES	6.1–7.5	6.76
MOPSO	6.2–7.6	6.90
Bis-Tris Propane	6.3–9.5	6.80 and 9.00
BES	6.4–7.8	7.09
MOPS	6.5–7.9	7.20
TES	6.8–8.2	7.40
HEPES	6.8–8.2	7.48
DIPSO	7.0–8.2	7.60
MOBS	6.9–8.3	7.60
TAPSO	7.0–8.2	7.60
Tris	7.0–9.0	8.06
HEPPSO	7.1–8.5	7.80
POPSO	7.2–8.5	7.80
TEA	7.3–8.3	7.80
EPPS	7.3–8.7	8.00
Tricine	7.4–8.8	8.05
Gly-Gly	7.5–8.9	8.20
Bicine	7.6–9.0	8.26
HEPBS	7.6–9.0	8.30
TAPS	7.7-9.1	8.40
AMPD	7.8–9.7	8.80
TABS	8.2–9.6	8.90
AMPSO	8.3–9.7	9.00
CHES	8.6–10.0	9.49
CAPSO	8.9–10.3	9.60
AMP	9.0–10.5	9.70
CAPS	9.7–11.1	10.40
CABS	10.0–11.4	10.70

BLENDED BUFFERS

One of the main benefits of blended buffers is that the target pH is reached without the addition of extra acid or base, which therefore prevents unnecessary conductivity in the solution. This can be important for ion exchange chromatography. In a manufacturing setting it also means that there are less additions to make.

To make a blended buffer using the various tables below:-

1. Select the buffer acid and base components you wish to use
2. Use the following calculation for each component (A & B) required for the buffer

Grams to weigh out = Molecular wt. x Target molarity (M) x Target volume (L) x for the desired pH take the % of component required from the relevant component ratio table for that buffer.

3. Add both components to the flask along with any other chemicals that are required for your buffer solution and make up to the target volume
4. Check pH with a calibrated pH probe

Example calculation

To make 2L of a 25mM (0.025M) Sodium Phosphate buffer at pH6.8

From

Table 6 **Component A** you decide to choose $Na_2HPO_4 \bullet 2H_2O$ with a mol. wt. of 178.05

The grams to weigh out = 178.05 x 0.025 x 2 x 0.2450 (from Table 7) = **2.18g**

From Table 6 **Component B** you decide to choose $NaH_2PO_4 \bullet H_2O$ with a mol. wt. of 137.99

The grams to weigh out = 137.99 x 0.025 x 2 x 0.2550 (from Table 7) = **1.76g**

ACETATE BUFFER: USEFUL RANGE PH3.7 TO 5.6

Table 2 : Acetate buffer components

Component	Name	Synonyms	Formula	Mol. Wt.
A	Sodium acetate	Acetic acid sodium salt	CH_3COONa	**82.03**
	Sodium acetate trihydrate	Acetic acid sodium salt trihydrate	$CH_3COONa \bullet 3H_2O$	**192.12**
B	Acetic acid *	N/A	CH_3COOH	**60.05**

* Note acetic acid is supplied as a solution. Therefore you need to calculate the volume to add to give you your target concentration. For example for a 2L solution of 25mM Acetate buffer at pH4.0 you would need 2.46g of acetic acid (60.05 x 0.025 x 2 x 0.82). If you have an 80% acetic acid stock solution you would need to add (100/80) x 2.46 = 3.08mL.

Table 3 : Acetate buffer component ratios for target pH

Target pH	% Component A (Sodium acetate)	% Component B (Acetic acid)
3.7	10.0	90.0
3.8	12.0	88.0
4.0	18.0	82.0
4.2	26.5	73.5
4.4	37.0	63.0
4.6	49.0	51.0
4.8	59.0	41.0
5.0	70.0	30.0
5.2	79.0	21.0
5.4	86.0	14.0
5.6	91.0	9.0

CITRATE BUFFER: USEFUL RANGE PH3.0 TO 6.2

Table 4 : Citrate buffer components

Component	Name	Synonyms	Formula	Mol. Wt.
A	Citric acid monohydrate	N/A	$C_6H_8O_7 \bullet H_2O$	**210.14**
	Or Citric acid	N/A	$C_6H_8O_7$	**192.12**
B	Trisodium citrate dihydrate	Sodium citrate tribasic dihydrate, Citric acid trisodium salt dihydrate	$C_6H_5O_7Na_3 \bullet 2H_2O$	**294.12**

Table 5 : Citrate buffer component ratios for target pH

Target pH	% Component A (Citric acid monohydrate or Citric acid)	% Component B (Trisodium citrate dihydrate)
3.0	82.0	18.0
3.2	77.5	22.5
3.4	73.0	27.0
3.6	68.5	31.5
3.8	63.5	36.5
4.0	59.0	41.0
4.2	54.0	46.0
4.4	49.5	50.5
4.6	44.5	55.5
4.8	40.0	60.0
5.0	35.0	65.0
5.2	30.5	69.5
5.4	25.5	74.5
5.6	21.0	79.0
5.8	16.0	84.0
6.0	11.5	88.5
6.2	8.0	92.0

SODIUM PHOSPHATE BUFFER: USEFUL RANGE PH5.8 TO 8.0

Table 6 : Sodium phosphate buffer components

Component	Name	Synonyms	Formula	Mol. Wt.
A	Sodium phosphate dibasic	Disodium hydrogen phosphate, Sodium hydrogen phosphate	Na_2HPO_4	**141.96**
	Or Sodium phosphate dibasic dihydrate	Disodium hydrogen phosphate dihydrate	$Na_2HPO_4 \bullet 2H_2O$	**178.05**
	Or Sodium phosphate dibasic heptahydrate	Disodium hydrogen phosphate heptahydrate	$Na_2HPO_4 \bullet 7H_2O$	**268.07**
	Or Sodium phosphate dibasic dodecahydrate	Disodium hydrogen phosphate dodecahydrate	$Na_2HPO_4 \bullet 12H_2O$	**358.22**
B	Sodium phosphate monobasic	Monosodium dihydrogen orthophosphate, Sodium dihydrogen phosphate	NaH_2PO_4	**119.98**
	Or Sodium phosphate monobasic monohydrate	Monosodium phosphate, Sodium dihydrogen phosphate monohydrate	$NaH_2PO_4 \bullet H_2O$	**137.99**
	Or Sodium phosphate monobasic dihydrate	Monosodium phosphate, Sodium dihydrogen phosphate dihydrate	$NaH_2PO_4 \bullet 2H_2O$	**156.03**

Table 7 : Sodium Phosphate buffer component ratios for target pH

Target pH	% Component A ($Na_2HPO_4 \bullet XH_2O$)	% Component B ($NaH_2PO_4 \bullet XH_2O$)
5.8	4.00	46.00
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	24.50	25.50
7.0	30.50	19.50
7.2	36.00	14.00
7.4	40.50	9.50
7.6	43.50	6.50
7.8	45.75	4.25
8.0	47.35	2.65

OTHER COMMON BUFFER RECIPES

PHOSPHATE BUFFERED SALINE A (PBS/A)

137mM Sodium chloride (NaCl)

2.7mM Potassium chloride (KCl)

10mM Disodium hydrogen phosphate (Na_2HPO_4)

1.8mM Potassium dihydrogen phosphate (KH_2PO_4)

pH adjust to 7.4 with 1M HCl

TRIS BUFFERED SALINE (TBS)

50mM Tris

150mM Sodium chloride (NaCl)

pH adjust to 7.5 with 1M HCl

REFERENCES

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[2] N. E. Good, G. D. Winget, W. Winter, T. N. Connolly, S. Izawa, and R. M. M. Singh, 'Hydrogen Ion Buffers for Biological Research *', *Biochemistry*, vol. 5, no. 2, pp. 467–477, Feb. 1966, doi: 10.1021/bi00866a011.

[3] C. M. H. Ferreira, I. S. S. Pinto, E. V. Soares, and H. M. V. M. Soares, '(Un)suitability of the use of pH buffers in biological, biochemical and environmental studies and their interaction with metal ions – a review', *RSC Adv.*, vol. 5, no. 39, pp. 30989–31003, 2015, doi: 10.1039/C4RA15453C.