Introduction

In the PACT Suite four buffer systems are used, each of which provides effective buffering across a broad pH range without changing the chemical composition of the buffering components. This simplifies analysis of results during optimization, since the pH of a solution can be varied but the buffer components used remain identical.

Recipe for SPG buffer stock solutions

SPG buffer is produced by mixing succinic acid, sodium dihydrogen phosphate, and glycine in the molar ratios 2:7:7 – succinic acid:sodium dihydrogen phosphate:glycine.* The three chemicals have three different buffering curves and their ratios have been selected to keep pH variation almost linear. The desired pH is obtained by mixing high- and low-pH stock solutions.

For a final volume of 100 ml:

1. **Weigh 1.48 g of succinic acid into an empty 100 ml beaker. Add 6.04 g sodium dihydrogen phosphate monohydrate and 3.28 g of glycine.**
2. **Add water to bring the volume to ~80 ml and stir until dissolved.**
3. **Adjust the pH to 4 or 9 by adding the appropriate amount 10 M NaOH.**
4. **Add water to complete the volume to 100 ml.**

   Use the resulting buffers as a 10x high-pH or low-pH stock solution for the SPG buffering system. A wide variety of pH values can be reached by mixing different ratios of high-pH and low-pH solution.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Recipe for MMT buffer stock solutions

MMT buffer is produced by mixing DL-malic acid, MES and Tris base in the molar ratios 1:2:2 – DL-malic acid:MES:Tris base.* Varying the amount of NaOH or HCl added, enables buffering over a pH range from 4–9. The three chemicals have three different buffering curves and their ratios have been selected to keep pH variation almost linear. The desired pH is obtained by mixing high- and low-pH stock solutions.

For a final volume of 100 ml:

1. **Weigh 2.68 g of DL-malic acid into an empty 100 ml beaker. Add 8.53 g MES monohydrate and 4.85 g of Tris base.**
2. **Add water to bring the volume to ~80 ml and stir until dissolved.**
3. **Adjust the pH to 4 by adding the appropriate amount 10 M HCl or 9 by adding the appropriate amount 10 M NaOH.**
4. **Add water to complete the volume to 100 ml.**

   Use the resulting buffers as a 10x high-pH or low-pH stock solution for the MMT buffering system. A wide variety of pH values can be reached by mixing different ratios of high-pH and low-pH solution.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Recipe for PCB (= PCTP) buffer stock solutions

PCB (= PCTP) buffer is produced by mixing sodium propionate, sodium cacodylate, and BIS-TRIS propane in the molar ratios 2:1:2 – sodium propionate, sodium cacodylate, and BIS-TRIS propane.* Varying the amount of HCl added enables buffering over a pH range from 4–9. The three chemicals have three different buffering curves and their ratios have been selected to keep pH variation almost linear. The desired pH is obtained by mixing high- and low-pH stock solutions.

For a final volume of 100 ml:

1. **Weigh 3.84 g of sodium propionate into an empty 100 ml beaker. Add 4.28 g sodium cacodylate trihydrate and 11.29 g of BIS-TRIS propane.**
2. **Add water to bring the volume to ~80 ml and stir until dissolved.**
3. **Adjust the pH to 4 or 9 by adding the appropriate amount 10 M HCl.**
4. **Add water to complete the volume to 100 ml.**
   Use the resulting buffers as a 10x high-pH or low-pH stock solution for the PCB (= PCTP) buffering system. A wide variety of pH values can be reached by mixing different ratios of high-pH and low-pH solution.

Recipe for MIB buffer stock solutions

MMT buffer is produced by mixing sodium malonate, imidazole, and boric acid in the molar ratios 2:3:3 – sodium malonate:imidazole:boric acid.* The three chemicals have three different buffering curves and their ratios have been selected to keep pH variation almost linear. The desired pH is obtained by mixing high- and low-pH stock solutions.

For a final volume of 100 ml:

1. **Weigh 4.15 g of sodium malonate dibasic monohydrate into an empty 100 ml beaker. Add 2.55 g imidazole and 2.32 g of boric acid.**
2. **Add water to bring the volume to ~80 ml and stir until dissolved.**
3. **Adjust the pH to 4 by adding the appropriate amount 10 M HCl or 9 by adding the appropriate amount 10 M NaOH.**
4. **Add water to complete the volume to 100 ml.**
   Use the resulting buffers as a 10x high-pH or low-pH stock solution for the MIB buffering system. A wide variety of pH values can be reached by mixing different ratios of high-pH and low-pH solution.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Molecular Dimensions

The Really Useful Buffer Kit MD2-100/MD2-101

Allows un-coupling of the chemical nature of the buffering components from pH.
8 broad-range buffering systems.

MD2-100 is presented as 16 x 50 mL buffers and MD2-101 is presented as 16 x 10 mL buffers

Features of The Really Useful Buffer Kit

- Buffer across a broad range without changing the chemical composition.
- Linear response to pH on mixing- vary pH.
- Un-couple the chemical nature for the buffering components from pH control.

Introduction

Buffers are commonly used in protein crystallization experiments: in the Jancarik and Kim screen (Jancarik & Kim, 1991) 39 of the 50 conditions contain a specific buffering chemical. The buffer component is often found at relatively high concentrations, most likely at 100mM. It can act both by modulating the pH of the protein solution during crystallization and as a chemical in the crystallization cocktail.

There are around 900 structures in the Protein Data Bank (PDB; Berman et al., 2000) that contain ordered MES, over 500 that contain ordered HEPES, and more than 900 that contain ordered Tris, showing that it is not unusual to have specific interactions between buffers and protein molecules. Table 1 shows a more extensive list of buffers found in structures deposited in the PDB.

Thus, a dilemma arises during optimization of how to uncouple the chemical nature and the buffering properties of the `buffer' component of a crystallization cocktail.

Traditionally, a crystallization laboratory will be stocked with all the common buffers, at many pH values within the buffers' useful buffering range. This allows for optimization of any given starting condition very rapidly. However, there is an increasing use of automation in crystallization, not only to set-up crystallization droplets, but also to prepare the arrays of crystallization cocktails used in the crystallization trials. Most of the liquid-handling robots that can be used to prepare crystallization cocktails are only able to handle a limited number of stock solutions at any one time

Buffer	PDB Code	Count
Succinic acid	SIN	160
Phosphate	PO4	4260
Glycine	GLY	112938
Citrate	CIT	688
HEPES	EPE	726
CHES	NHE	124
Malonate	MLT	78
Imidazole	IMD	546
Borate	BO3	16
Propanoic acid	PPI	29
Cacodylate	CAD	51
Acetate	ACT	3579
ADA	MHA	4
Bicine	BCN	52
MES	MES	924
Tris	TRS	913
Tartrate	TLA	295
Bis-Tris	BTB	136

Table 1. Buffers found in the systems and their frequency in the PDB (March, 2016).

These numbers were obtained by using the HIC-Up (Kleywegt & Jones, 1998) site to identify a PDB code and then searching the PDB for instances of files containing those codes. In some cases the PDB was searched directly, using the `Search by Ligands' function.

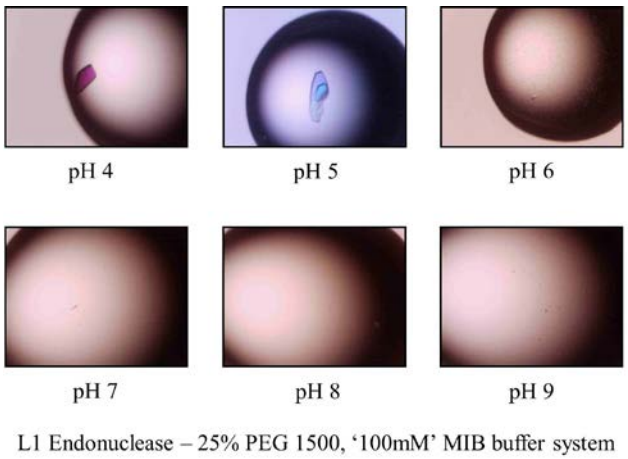

Figure 1

Molecular Dimensions

Using broad-range buffers

To address this challenge, Newman (Newman, J., 2004) has developed a series of buffers by mixing different buffering components. This set of buffers, complimented by the imidazole-malate buffer from our Stura and Macrosol screens, is now presented as a kit.

Each pair from this series of broad-range buffers permits a wide range of pH values to be set-up, thus allowing the pH to be changed without altering the buffer chemistry. Each broad-range system consists of component buffers chosen to have a variety of chemistries. This allows one to increase both the pH range of the component buffers and the chemical variability of any screen or optimization strategy that incorporates these buffer systems. These buffers are ideal for applications where the number of stock solutions which can be used is limited, as is the case with most liquid-handling robots.

Using only 16 stock solutions any number of different pH values can be achieved within the given range for each of the eight buffer systems. Preliminary testing of the buffer systems suggests that these systems are compatible with other common protein crystallization reagents.

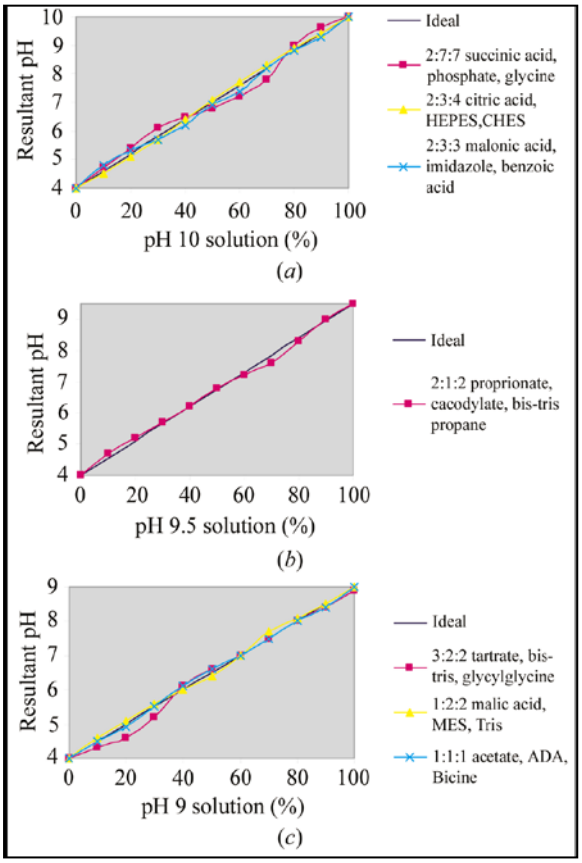

Figure 2

a) Three buffer systems that span the pH range 4.0±10.0, (b) a buffer system that covers the pH range 4.0±9.5 and (c) buffer systems that cover the pH range 4.0±9.0. The correlation coefficients of the lines of best fit range from 0.985 (succinic acid, phosphate, glycine) to 0.999 (citric acid, HEPES, CHES).

Suggestions for use

The ratios of the components within the first seven of these eight buffer systems have been carefully selected so as to produce a reasonably linear response to pH, so that if the low pH stock is at pH 4 and the high pH stock is at pH 10, then a 3:1 mix of these two stocks would yield a solution of pH 5.5, a 1:1 mix would result in a pH 7 and a 1:3 ratio would give a solution of pH 8.5. This is illustrated in the graphs opposite (after Newman, 2004).

Being only a two component buffer the imidazole-malate buffer is not prepared in this way and the two components must be titrated against each other to achieve a desired pH.

The most economical way of setting up a series of buffers would be to titrate one stock with the other, removing an aliquot at each desired pH. Each aliquot can then be used with other reagents to make up final screen conditions diluted to final molarities.

Table 2. Guidelines for Preparation of Buffer Components

For MIB and CHC buffers		
Desired pH (approx.)	Volume of pH 4	Volume of pH 10
4	1000	0
5	835	165
6	670	330
7	500	500
8	330	670
9	165	835
10	0	1000
For PCTP (PCB) buffer		
Desired pH (approx.)	Volume of pH 4	Volume of pH 9.5
4	1000	0
5	835	165
6	670	330
7	500	500
8	330	670
9	165	835
9.5	0	1000
For SPG Buffer		
Desired pH (approx.)	Volume of pH 4	Volume of pH 10
4	1000	0
5	860	140
6	670	330
7	420	580
8	270	730
9	200	800
10	0	1000
For MMT and AAB Buffers		
Desired pH (approx.)	Volume of pH 4	Volume of pH 9
4	1000	0
5	800	200
6	600	400
7	400	600
8	200	800
9	0	1000
For TBG Buffer		
Desired pH (approx.)	Volume of pH 4	Volume of pH 9
4	1000	0
5	760	240
6	680	320
7	500	500
8	250	750
9	0	1000

Table 2 contd.

1M Imidazole/1M Malic Acid

Following values given below are approximate and may vary according to your pH meter.

pH	Imidazole(56750) starting volume (mL)	Malic Acid (M0875) volume added (mL)	% Imidazole	% Malic Acid
9	100	1	99	1
8.5	100	2.5	98.5	2.5
8.2	100	5	95	5
8	100	7.5	93	7
7.8	100	11	90	10
7.5	100	17.5	85	15
7.2	100	25	80	20
7	100	30	77	23
6.8	100	35	74	26
6.5	100	42	70	30
6	100	48	68	32
5.7	100	50	67	33
5.5	100	52	66	34
5.2	100	55	64.5	35.5
5	100	58	63.3	36.7
4.8	100	62	62	38
4.5	100	71	58.5	41.5
4.2	100	83	55	45
4	100	94	51.5	48.5

Initial pH

	pH
1 M Malic Acid	4
1 M Imidazole	10

The Really Useful Buffer Kit MD2-100/MD2-101

Bottle		pH	Chemical	Ratio*
1	1M SPG	4	Succinic acid	2
2	1M SPG	10	Sodium dihydrogen phosphate monohydrate	7
			Glycine	7
3	1M MIB	4	Sodium malonate	2
4	1M MIB	10	Imidazole	3
			Boric acid	3
5	1M PCTP	4	Sodium propionate	2
6	1M PCTP	9.5	Sodium cacodylate trihydrate	1
			Bis-Tris propane	2
7	1M MMT	4	DL-Malic acid	1
8	1M MMT	9	MES monohydrate	2
			Trizma® base	2
9	1M CHC	4	Citric acid	2
10	1M CHC	10	HEPES	3
			CHES	4
11	1M AAB	4	Sodium acetate trihydrate	1
12	1M AAB	9	ADA	1
			BICINE	1
13	1M TBG	4	Sodium tartrate	3
14	1M TBG	9	Bis-Tris	2
			Gly-Gly	2
15	1M		Imidazole	
16	1M		DL-Malic acid	

*The ratios of the components are listed on the datasheet for the Really Useful Buffer Kit, and the concentrations are set to give a notional 1 molar solution. E.g. if the components are in the ratio 2 parts A: 7 parts B: 7 parts C; then the concentrations are 2/16M A; 7/16M B and 7/16M C.

Molecular Dimensions

Formulation Notes:

The Really Useful Buffer reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents. Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com
Enquiries regarding the really Useful Buffer Kit formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

References

1. Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. & Bourne, P. E. (2000). Nucleic Acids Res. 28, 235- 242.
2. Jancarik, J. & Kim, S.-H. (1991). J. Appl. Cryst. 24, 409-411.
3. Kleywegt, G. J. & Jones, T. A. (1998). Acta Cryst. D54, 1119-1131.
4. Newman, J. (2004) Acta Cryst D60, 610-612.

Re-Ordering details:

Catalogue Description	Pack size*	Catalogue Code
The Really Useful Buffer Kit	16 x 50 mL	MD2-100
The Really Useful Buffer Kit	16 x 10 mL	MD2-101
Single Reagents		
1M SPG Buffer pH 4 and pH 10	2 x 100 mL	MD2-59
1M MIB Buffer pH 4 and pH 10	2 x 100 mL	MD2-60
1M PCTP Buffer pH 4 and pH 9.5	2 x 100 mL	MD2-61
1M MMT Buffer pH 4 and pH 9	2 x 100 mL	MD2-62
1M CHC Buffer pH 4 and pH 9	2 x 100 mL	MD2-63
1M AAB Buffer pH 4 and pH 9	2 x 100 mL	MD2-64
1M TBG Buffer pH 4 and pH 9	2 x 100 mL	MD2-65
1M Imidazole and 1M DL-Malic acid	2 x 100 mL	MD2-66

*other volumes can be ordered- please e-mail us at enquiries@moleculardimensions.com for custom volumes.