Molecular Dimensions

Soluble Proteins

Morpheus® MD1–46

A 96 condition, 3D protein crystallization screen incorporating a range of low-molecular weight ligands. Unlock novel chemical space previously inaccessible using conventional screens.

MD1-46 is presented as 96 x 10 mL conditions.

Features of Morpheus®:

- Simple and effective 3D grid design covering a range of pH, precipitants, PEGs and salt additives.
- Targeted incorporation of 49 low molecular weight ligands.
- Morpheus® ligands promote initial crystal formation and lattice stability.
- Reduced crystal "stress" – all conditions are cryoprotected*.
- Easy optimization of 'hits'.
- Readily available Morpheus® Optimization reagents including the Mixes and Stock reagents.

Introduction

Morpheus®**(1)** is a 96 condition protein crystallization screen with an original chemistry. It is based on extensive data mining of the PDB. The aim is to explore different chemical space than is achieved with conventional screening.

Morpheus® incorporates 49 low molecular weight components. They are PDB ligands sharing four main characteristics; they are small (the largest being HEPES MW 238.30 g/mol and the smallest a lithium ion MW 6.94 g/mol), stable, inexpensive and are associated with at least five unrelated PDB structures.

The selection of ligands is listed in Table 1 (data produced on the 14th of July 2008: 35759 structures with ligands in the PDB). Overall the PDB ligands in Morpheus® correspond with over 33,000 PDB structures. For instance, the two enantiomers of tartaric acid (PDB ID: TAR and TLA) are found ordered in 113 structures.

Preliminary tests with Morpheus® made within the Laboratory of Molecular Biology (LMB)[1] at Cambridge, UK, have shown encouraging results with various targets. In some cases, Morpheus® gave hits when all other commercial screens had failed.

Figure 1 shows examples of protein crystallization hits observed while testing Morpheus.

*All the conditions of Morpheus are to some extent cryo-protected to minimize further mechanical stress on the crystals. For example, all PEG 4000 conditions contain a suitable amount of Glycerol.

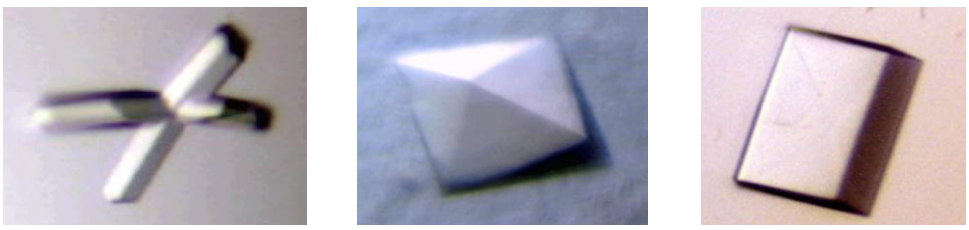

Figure 1. Examples of successful crystallization with Morpheus®
(with the permission of Pobbati A., Low H. and Berndt A.)

Screen Design

Morpheus is based on a 3D grid design (Figure 2). Thirty of the top PDB ligands from Table 1 are grouped into eight mixes of additives depending on their chemical class (e.g. alcohols, carboxylic acids, etc) (Table 2).

These top PDB ligands also happen to be "biological buffers" like HEPES (PDB ID: EPE, 201 hits) and have been used to build three buffer systems*. Each buffer system includes different buffers with close pKa's (Table 3).

There are nine precipitants included in the composition of Morpheus. They are grouped into four mixes of precipitants (Table 4). The main characteristic of the four mixes is that they contain at least a PEG (Polyethylene glycol) and a different type of precipitant that is also a cryo-agent (e.g. Glycerol). All the conditions of Morpheus are cryo-protected to minimize further mechanical stress on the crystals.

Each mix of precipitants is systematically tested with all the mixes of additives and the mixes of buffers. The proportions of stocks are always the same for making any condition of the three-dimensional grid: 5:1:1:3 of precipitants, ligands, buffers and water respectively. When almost a third of the composition is water, there is space for making an optimization screen with higher concentration of any mix/component.

References

1. Gorrec, F. (2009). J. Appl. Cryst. 42, 1035-1042.

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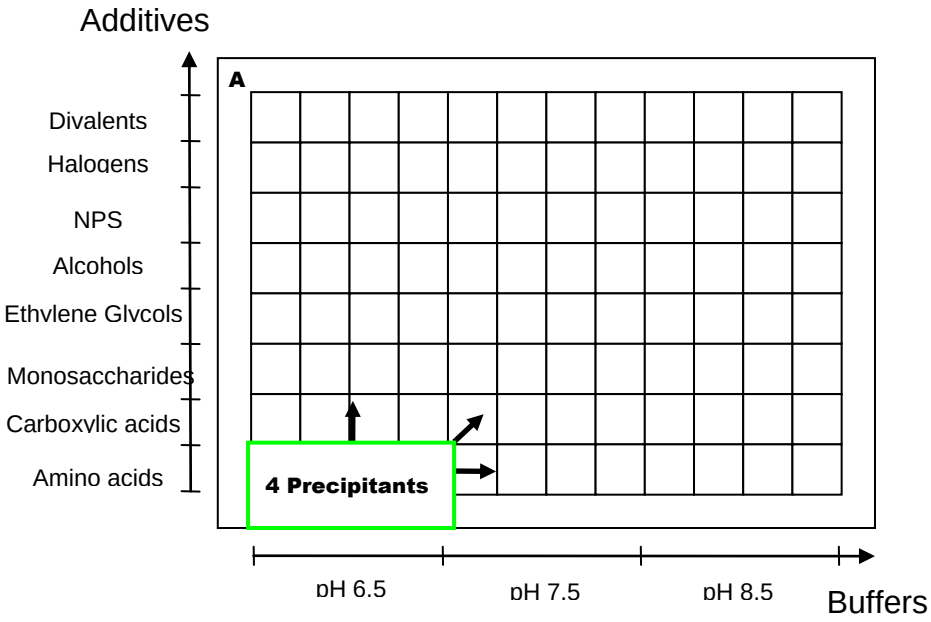


Figure 2. Schematic of Morpheus® – A three-dimensional grid screen

Formulation Notes:

Morpheus® 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.

Enquiries regarding Morpheus® formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com

Morpheus Optimization

There are two main things to consider in first instance optimizing hits with Morpheus:

Although the screen is composed of various mixes, consider a condition like in any other screen, with three stock solutions:

- mix of precipitants
- mix of salts (here called additives)
- mix of buffers.

When you have more than one hit, you can deduce the importance of each stock right from the beginning: e.g. Do I see specificity related to one stock? to pH?

Stocks are made in such way there are 2x [Precipitant], 10x [Salt], 10x [Buffer] compared to the final concentrations in the screen.
You can make optimization 2D grid screens, by varying the stock concentrations.
To vary the pH, you can change the ratio of the two buffers within the buffer stock (i.e. change ratio of two non-titrated 1M buffer stocks).
Once you know more about the chemical space within Morpheus you can eventually push the investigation further, trying to reduce the number of chemicals.
For example, what happens when you replace the group of chemicals from a stock with only one chemical of this mix? (e.g. only PEG instead of PEG + Glycerol).

At this stage you may (or not) have a simpler condition to work with. In any case, you have explored all the potential of Morpheus itself, and you can proceed to other "classic" optimization approaches such as using additives, or seeding.

Table 1: List of PDB ligands in Morpheus®

PDB Ligand name(s)	Class	PDB ID(s)	Structure Hits*
1,2-Ethanediol (ethylene glycol)	Precipitant	EDO, EGL	1081
1,2-Propanediol (enantiomers R and S)	Alcohols	PGO, PGR	41
1,3-Propanediol	Alcohols	PDO	7
1,4-Butanediol	Alcohols	BU1	11
1,6-Hexanediol	Alcohols	HEZ	19
1-Butanol	Alcohols	1BO	7
2-(N-Morpholino)-ethane sulfonic acid (MES)	Buffer	MES	315
2-Amino-2-hydroxymethyl-propane-1,3-diol (Tris)	Buffer	TRS	334
2-Methyl-2,4-pentanediol (MPD, enantiomers R and S)	Precipitant	MPD, MRD	504
3-Morpholinopropane-1-sulfonic acid (MOPS)	Buffer	MPO	21
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Buffer	EPE	201
Acetic acid, acetate, acetyl	Carboxylic acids	ACY, ACT, ACE	1890
(*S*)-2-Aminopropanoic acid (Alanine, (enantiomers L and D)	Amino acids	ALA, DAL	35
Amino, Ammonia, Ammonium	multiple	NH2, NH3, NH4	582
N,N-bis(2-hydroxyethyl)glycine (Bicine)	Buffer	BCN	13
Bromide	Halogens	BR	120
Calcium	Divalents	CA	3959
Chloride	Multiple	CL	2842
Citric acid, citrate	Carboxylic acids	CIT, FLC	384
D-Galactose (anomers α and β)	Monosaccharides	GAL, GLA	86
D-Glucose (anomers α and β)	Monosaccharides	GLC, BGC	206
Di(Hydroxyethyl)ether (Di-Ethyleneglycol)	Ethylene glycols	PEG	209
D-Mannose (anomers α and β)	Monosaccharides	MAN, BMA	178
D-Xylopyranose (anomers α and β)	Monosaccharides	XYL, XYP	41
Fluoride	Halogens	F	16
Formic acid	Carboxylic acids	FMT	267
Glycerol	Amino acids	GOL	2884
Glycine	Buffer	GLY	50
Imidazole	Halogens	IMD	154
Iodide	Alcohols	IOD	178
Isopropyl alcohol (iso-propanol, 2-Propanol)	Monosaccharides	IPA, IOH	174
L-Fucose (anomers α and β)	Amino acids	FUC, FUL	62
L-Glutamic acid	Precipitant	GLU	66
Lysine (enantiomers L and D)	Amino acids	LYS, DLY	36
Magnesium	Divalents	MG	3991
N-Acetyl-d-glucosamine (anomers α and β)	Monosaccharides	NAG,NBG	1150
Nitrate	NPS	NO3	156
Oxamic acid	Carboxylic acids	OXM	17
Penta(hydroxyethyl)ether (Penta-Ethyleneglycol)	Ethylene glycols	1PE	91
Phosphates	NPS	PO4, PI, 2HP	1687
Potassium	Carboxylic acids	K	720
Serine (enantiomers L and D)	Amino acids	SER, DSN	38
Sodium	multiple	NA	1926
Sulfate	NPS	SO4	5793
Tartaric acid (enantiomers R and S)	Carboxylic acids	TAR, TLA	113
Tetra(hydroxyethyl)ether (Tetra-Ethyleneglycol)	Ethylene glycols	PG4	194
Tri(Hydroxyethyl)ether (Tri-Ethyleneglycol)	Ethylene glycols	PGE	107
* as of July 2008.		**SUM**	32956

Table 2: **Mixes of additives used in Morpheus®**

Mix name	Composition	Catalogue Number (100 & 250mL)
Divalents	$MgCl_2$; $CaCl_2$	MD2-100(250)-70
Halogens	NaF; NaBr; NaI	MD2-100(250)-71
NPS†	$NaNO_3$; Na_2HPO_4; $(NH_4)_2SO_4$	MD2-100(250)-72
Alcohols	1,6-Hexanediol; 1-Butanol 1,2-Propanediol (racemic); 2-Propanol; 1,4-Butanediol; 1,3-Propanediol	MD2-100(250)-73
Ethylene glycols	Di-Ethyleneglycol; Tri-Ethyleneglycol; Tetra-Ethyleneglycol; Penta-Ethyleneglycol	MD2-100(250)-74
Monosaccharides	D-Glucose; D-Mannose; D-Galactose; L-Fucose; D-Xylose; N-Acetyl-D-Glucosamine	MD2-100(250)-75
Carboxylic acids	Na-Formate; NH_4-Acetate; Na_3-Citrate; NaK-Tartrate (racemic); Na-Oxamate	MD2-100(250)-76
Amino acids	L-Na-Glutamate; Alanine (racemic); Glycine; Lysine HCl (racemic); Serine (racemic)	MD2-100(250)-77

†**NPS;** Nitrate Phosphate Sulfate

Table 3: **Buffer systems used in Morpheus®**

Mix name	Conc.	pH @ 20°C	Composition	Catalogue Number (100 & 250mL)
Buffer System 1	1.0M	6.5	Imidazole; MES (acid)	MD2-100(250)-100
Buffer System 2	1.0M	7.5	Sodium HEPES; MOPS (acid)	MD2-100(250)-101
Buffer System 3	1.0M	8.5	Tris (base); Bicine	MD2-100(250)-102

Table 4: **Mixes of Precipitants used in Morpheus®**

Mix name	Conc.	Composition	Catalogue Number (100 & 250mL)
P500MME_P20K	60%	PEG 500* MME; PEG 20K	MD2-100(250)-81
EDO_P8K	60%	Ethylene glycol; PEG 8K	MD2-100(250)-82
GOL_P4K	60%	Glycerol; PEG 4K	MD2-100(250)-83
MPD_P1K_P3350	75%	MPD (racemic); PEG 1K; PEG 3350	MD2-100(250)-84

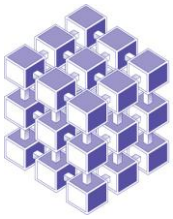
*N.B. The PEG 550 MME that was originally used in this screen has been discontinued and replaced with PEG 500 MME.

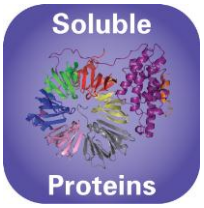
Molecular Dimensions

Soluble Proteins

Morpheus® Conditions 1-48 (Box 1) MD1–46

Tube	pH	Conc	Ligands	Conc	Buffer	Conc	Precipitant
1-1	6.5	0.06 M	Divalents	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
1-2	6.5	0.06 M	Divalents	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
1-3	6.5	0.06 M	Divalents	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
1-4	6.5	0.06 M	Divalents	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
1-5	7.5	0.06 M	Divalents	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
1-6	7.5	0.06 M	Divalents	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
1-7	7.5	0.06 M	Divalents	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
1-8	7.5	0.06 M	Divalents	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
1-9	8.5	0.06 M	Divalents	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
1-10	8.5	0.06 M	Divalents	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
1-11	8.5	0.06 M	Divalents	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
1-12	8.5	0.06 M	Divalents	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350
1-13	6.5	0.09 M	Halogens	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
1-14	6.5	0.09 M	Halogens	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
1-15	6.5	0.09 M	Halogens	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
1-16	6.5	0.09 M	Halogens	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
1-17	7.5	0.09 M	Halogens	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
1-18	7.5	0.09 M	Halogens	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
1-19	7.5	0.09 M	Halogens	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
1-20	7.5	0.09 M	Halogens	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
1-21	8.5	0.09 M	Halogens	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
1-22	8.5	0.09 M	Halogens	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
1-23	8.5	0.09 M	Halogens	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
1-24	8.5	0.09 M	Halogens	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350
1-25	6.5	0.09 M	NPS	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
1-26	6.5	0.09 M	NPS	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
1-27	6.5	0.09 M	NPS	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
1-28	6.5	0.09 M	NPS	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
1-29	7.5	0.09 M	NPS	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
1-30	7.5	0.09 M	NPS	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
1-31	7.5	0.09 M	NPS	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
1-32	7.5	0.09 M	NPS	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
1-33	8.5	0.09 M	NPS	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
1-34	8.5	0.09 M	NPS	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
1-35	8.5	0.09 M	NPS	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
1-36	8.5	0.09 M	NPS	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350
1-37	6.5	0.12 M	Alcohols	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
1-38	6.5	0.12 M	Alcohols	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
1-39	6.5	0.12 M	Alcohols	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
1-40	6.5	0.12 M	Alcohols	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
1-41	7.5	0.12 M	Alcohols	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
1-42	7.5	0.12 M	Alcohols	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
1-43	7.5	0.12 M	Alcohols	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
1-44	7.5	0.12 M	Alcohols	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
1-45	8.5	0.12 M	Alcohols	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
1-46	8.5	0.12 M	Alcohols	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
1-47	8.5	0.12 M	Alcohols	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
1-48	8.5	0.12 M	Alcohols	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350





Morpheus® Conditions 1-48 (Box 2) MD1–46

Tube	pH	Conc	Ligands	Conc	Buffer	Conc	Precipitant
2-1	6.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
2-2	6.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
2-3	6.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
2-4	6.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
2-5	7.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
2-6	7.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
2-7	7.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
2-8	7.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
2-9	8.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
2-10	8.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
2-11	8.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
2-12	8.5	0.12 M	Ethylene Glycols	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350
2-13	6.5	0.12 M	Monosaccharides	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
2-14	6.5	0.12 M	Monosaccharides	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
2-15	6.5	0.12 M	Monosaccharides	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
2-16	6.5	0.12 M	Monosaccharides	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
2-17	7.5	0.12 M	Monosaccharides	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
2-18	7.5	0.12 M	Monosaccharides	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
2-19	7.5	0.12 M	Monosaccharides	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
2-20	7.5	0.12 M	Monosaccharides	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
2-21	8.5	0.12 M	Monosaccharides	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
2-22	8.5	0.12 M	Monosaccharides	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
2-23	8.5	0.12 M	Monosaccharides	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
2-24	8.5	0.12 M	Monosaccharides	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350
2-25	6.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
2-26	6.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
2-27	6.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
2-28	6.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
2-29	7.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
2-30	7.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
2-31	7.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
2-32	7.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
2-33	8.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
2-34	8.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
2-35	8.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
2-36	8.5	0.1 M	Carboxylic acids	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350
2-37	6.5	0.1 M	Amino acids	0.1 M	Buffer System 1	30.00% % v/v	P500MME_P20K
2-38	6.5	0.1 M	Amino acids	0.1 M	Buffer System 1	30.00% % v/v	EDO_P8K
2-39	6.5	0.1 M	Amino acids	0.1 M	Buffer System 1	30.00% % v/v	GOL_P4K
2-40	6.5	0.1 M	Amino acids	0.1 M	Buffer System 1	37.50% % v/v	MPD_P1K_P3350
2-41	7.5	0.1 M	Amino acids	0.1 M	Buffer System 2	30.00% % v/v	P500MME_P20K
2-42	7.5	0.1 M	Amino acids	0.1 M	Buffer System 2	30.00% % v/v	EDO_P8K
2-43	7.5	0.1 M	Amino acids	0.1 M	Buffer System 2	30.00% % v/v	GOL_P4K
2-44	7.5	0.1 M	Amino acids	0.1 M	Buffer System 2	37.50% % v/v	MPD_P1K_P3350
2-45	8.5	0.1 M	Amino acids	0.1 M	Buffer System 3	30.00% % v/v	P500MME_P20K
2-46	8.5	0.1 M	Amino acids	0.1 M	Buffer System 3	30.00% % v/v	EDO_P8K
2-47	8.5	0.1 M	Amino acids	0.1 M	Buffer System 3	30.00% % v/v	GOL_P4K
2-48	8.5	0.1 M	Amino acids	0.1 M	Buffer System 3	37.50% % v/v	MPD_P1K_P3350

Manufacturer's safety data sheets are available from our website or by scanning the QR code here:

Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
Morpheus®	96 x 10 mL	MD1-46
Morpheus® HT-96	96 x 1 mL	MD1-47
Morpheus® single reagents	100 mL	MDSR-46-tube number
Morpheus® single reagents	100 mL	MDSR-47-well number
Morpheus® Additive OptiMax Kit	43 x 10 mL	MD1-58

All Morpheus® Mixes are available to buy from Molecular Dimensions Ltd.