



Morpheus® 10 mL, HT-96 and FX-96 pre-filled plate MD1-46, MD1-47 and MD1-47-FX

Morpheus is 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. MD1-47 is presented as 96 x 1 mL conditions. MD1-47-FX is presented as 96 x 100 µL 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.
- Suitable for membrane proteins with PEGs and polyols as main precipitants.
- 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® 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.

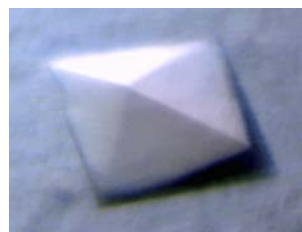


Figure 1. Examples of successful crystallization with Morpheus® (with the permission of Pobbati A., Low H. and Berndt A.)



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) The MORPHEUS protein crystallization screen *J Appl Cryst* **42**, 1035-1042.

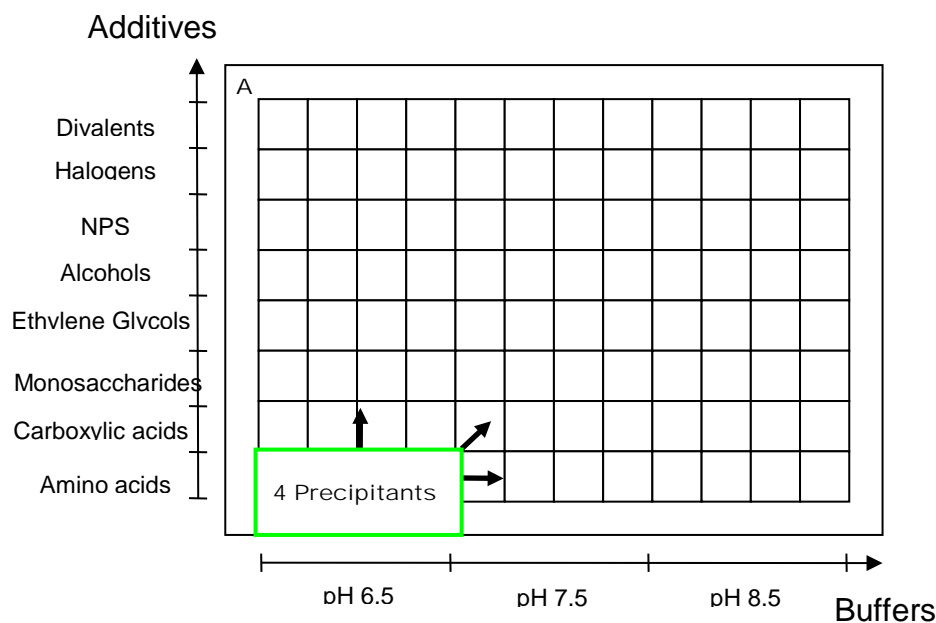


Figure 2. Schematic of Morpheus® – A three-dimensional grid screen



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

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



Morpheus Optimization

Although the screen is composed of various mixes, consider each condition as for any other screen, with three stock solutions:

- mix of precipitants
- mix of additives
- mix of buffers.

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

Every condition can be made following the same ratio of stock solutions:

$1/2$ [Precipitant mix] + $1/10$ [additive mix] + $1/10$ [Buffer system] + $3/10$ dH₂O.

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 investigate further, trying to reveal specificity of a single chemical.

For example, what happens when you replace the group of chemicals from a stock with only one chemical of this mix? (e.g. only one divalent cations instead of the corresponding mix of additives).

At this stage you may (or not) have a simpler condition to work with. You can also proceed to other "classic" optimization approaches such as using an additive screen, scale-up or seeding.



Table 1: List of PDB ligands in Morpheus®

| PDB Ligand name(s) | Class | PDB ID(s) | Number of Structures* |
|--|------------------|---------------|-----------------------|
| 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 |
| Glutamic acid (enantiomers L and D) | Precipitant | GLU, DGL | 75 |
| 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 |
| 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 |
| | SUM | | 32956 |

*As determined by querying the PDB in July 2008

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

| Mix name | Composition | Catalogue Number (100 mL) | Catalogue Number (250 mL) |
|------------------|--|---------------------------|---------------------------|
| Divalents | 0.3M Magnesium chloride hexahydrate; 0.3M Calcium chloride dihydrate | MD2-100-70 | MD2-250-70 |
| Halogens | 0.3M Sodium fluoride; 0.3M Sodium bromide; 0.3M Sodium iodide | MD2-100-71 | MD2-250-71 |
| NPS [†] | 0.3M Sodium nitrate, 0.3 Sodium phosphate dibasic, 0.3M Ammonium sulfate | MD2-100-72 | MD2-250-72 |
| Alcohols | 0.2M 1,6-Hexanediol; 0.2M 1-Butanol 0.2M 1,2-Propanediol; 0.2M 2-Propanol; 0.2M 1,4-Butanediol; 0.2M 1,3-Propanediol | MD2-100-73 | MD2-250-73 |
| Ethylene glycols | 0.3M Diethylene glycol; 0.3M Triethylene glycol; 0.3M Tetraethylene glycol; 0.3M Pentaethylene glycol | MD2-100-74 | MD2-250-74 |
| Monosaccharides | 0.2M D-Glucose; 0.2M D-Mannose; 0.2M D-Galactose; 0.2M L-Fucose; 0.2M D-Xylose; 0.2M N-Acetyl-D-Glucosamine | MD2-100-75 | MD2-250-75 |
| Carboxylic acids | 0.2M Sodium formate; 0.2M Ammonium acetate; 0.2M Sodium citrate tribasic dihydrate; 0.2M Potassium sodium tartrate tetrahydrate; 0.2M Sodium oxamate | MD2-100-76 | MD2-250-76 |
| Amino acids | 0.2M DL-Glutamic acid monohydrate; 0.2M DL-Alanine; 0.2M Glycine; 0.2M DL-Lysine monohydrochloride; 0.2M DL-Serine | MD2-100-77 | MD2-250-77 |

[†]NPS; Nitrate Phosphate Sulfate**Table 3: Buffer systems used in Morpheus®**

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

Table 4: Mixes of Precipitants used in Morpheus®

| Mix name | Old Mix Name | Composition | Catalogue Number (100 mL) | Catalogue Number (250 mL) |
|-----------------------|---------------|---|---------------------------|---------------------------|
| 60% Precipitant Mix 1 | P500MME_P20K | 40% v/v PEG 500* MME; 20 % w/v PEG 20000 | MD2-100-81 | MD2-250-81 |
| 60% Precipitant Mix 2 | EDO_P8K | 40% v/v Ethylene glycol; 20 % w/v PEG 8000 | MD2-100-82 | MD2-250-82 |
| 60% Precipitant Mix 3 | GOL_P4K | 40% v/v Glycerol; 20% w/v PEG 4000 | MD2-100-83 | MD2-250-83 |
| 75% Precipitant Mix 4 | MPD_P1K_P3350 | 25% v/v MPD; 25% PEG 1000; 25% w/v PEG 3350 | MD2-100-84 | MD2-250-84 |

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

Manufacturer's safety data sheets are available from our website.



RE-ORDERING
INFORMATION

| Code | Pack Size | Description |
|---|-------------|---|
| MD1-46 | 96 x 10 mL | Morpheus |
| MD1-47 | 96 x 1 mL | Morpheus HT-96 |
| MD1-47-FX | 96x 100 µL | Morpheus FX-96 pre-filled plate |
| Other Morpheus screens | | |
| MD1-91 | 96 x 10 mL | Morpheus II |
| MD1-92 | 96 x 1 mL | Morpheus II HT-96 |
| MD1-92-FX | 96x 100 µL | Morpheus II FX-96 pre-filled plate |
| MD1-93 | 48 x 100 µL | The Morpheus® Additive screen |
| MD1-116 | 96 x 10 mL | Morpheus III |
| MD1-117 | 96 x 1 mL | Morpheus III HT-96 |
| MD1-118 | 48 x 100 µL | Hippocrates™ additive screen |
| Green screens (contain green fluorescent dye - ideal for UV) | | |
| MD1-46-GREEN | 96 x 10 mL | Morpheus Green screen |
| MD1-47-GREEN | 96 x 1 mL | Morpheus HT-96 Green screen |
| Combo Packs | | |
| MD1-76 | 192 x 10 mL | Power combo value pack (Morpheus + MIDASplus) |
| MD1-76-HT | 192 x 1 mL | Power combo value pack HT-96 (Morpheus + MIDASplus HT-96) |
| Single reagents | | |
| MDSR-46-tube number | 100 mL | Morpheus single reagents |
| MDSR-47-well number | 100 mL | Morpheus HT-96 single reagents |

Try our newest screen Morpheus® III
MD1-116 (10 mL) and MD1-117 (HT-96)
for even more success with your crystallisations

Morpheus, Morpheus II and Morpheus III have been designed and developed by Fabrice GORREC, in collaboration with the scientists at the Medical Research Council Laboratory of Molecular Biology (LMB) at Cambridge and is manufactured exclusively under license from LifeARC by Molecular Dimensions Limited.

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| Screen ID | Well # | Conc | Ligands | Conc | Buffer | pH | Conc | Precipitant |
|-----------|--------|--------|-----------|-------|-----------------|-----|------------|-------------------|
| 1-1 | A1 | 0.06 M | Divalents | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 1-2 | A2 | 0.06 M | Divalents | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 1-3 | A3 | 0.06 M | Divalents | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 1-4 | A4 | 0.06 M | Divalents | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-5 | A5 | 0.06 M | Divalents | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 1-6 | A6 | 0.06 M | Divalents | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 1-7 | A7 | 0.06 M | Divalents | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 1-8 | A8 | 0.06 M | Divalents | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-9 | A9 | 0.06 M | Divalents | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 1-10 | A10 | 0.06 M | Divalents | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 1-11 | A11 | 0.06 M | Divalents | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 1-12 | A12 | 0.06 M | Divalents | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-13 | B1 | 0.09 M | Halogens | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 1-14 | B2 | 0.09 M | Halogens | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 1-15 | B3 | 0.09 M | Halogens | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 1-16 | B4 | 0.09 M | Halogens | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-17 | B5 | 0.09 M | Halogens | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 1-18 | B6 | 0.09 M | Halogens | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 1-19 | B7 | 0.09 M | Halogens | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 1-20 | B8 | 0.09 M | Halogens | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-21 | B9 | 0.09 M | Halogens | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 1-22 | B10 | 0.09 M | Halogens | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 1-23 | B11 | 0.09 M | Halogens | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 1-24 | B12 | 0.09 M | Halogens | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-25 | C1 | 0.09 M | NPS | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 1-26 | C2 | 0.09 M | NPS | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 1-27 | C3 | 0.09 M | NPS | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 1-28 | C4 | 0.09 M | NPS | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-29 | C5 | 0.09 M | NPS | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 1-30 | C6 | 0.09 M | NPS | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 1-31 | C7 | 0.09 M | NPS | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 1-32 | C8 | 0.09 M | NPS | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-33 | C9 | 0.09 M | NPS | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 1-34 | C10 | 0.09 M | NPS | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 1-35 | C11 | 0.09 M | NPS | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 1-36 | C12 | 0.09 M | NPS | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-37 | D1 | 0.12 M | Alcohols | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 1-38 | D2 | 0.12 M | Alcohols | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 1-39 | D3 | 0.12 M | Alcohols | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 1-40 | D4 | 0.12 M | Alcohols | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-41 | D5 | 0.12 M | Alcohols | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 1-42 | D6 | 0.12 M | Alcohols | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 1-43 | D7 | 0.12 M | Alcohols | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 1-44 | D8 | 0.12 M | Alcohols | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 1-45 | D9 | 0.12 M | Alcohols | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 1-46 | D10 | 0.12 M | Alcohols | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 1-47 | D11 | 0.12 M | Alcohols | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 1-48 | D12 | 0.12 M | Alcohols | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |



| Tube | Well | Conc | Ligands | Conc | Buffer | pH | Conc | Precipitant |
|------|------|--------|------------------|-------|-----------------|-----|------------|-------------------|
| 2-1 | E1 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 2-2 | E2 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 2-3 | E3 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 2-4 | E4 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-5 | E5 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 2-6 | E6 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 2-7 | E7 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 2-8 | E8 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-9 | E9 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 2-10 | E10 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 2-11 | E11 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 2-12 | E12 | 0.12 M | Ethylene glycols | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-13 | F1 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 2-14 | F2 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 2-15 | F3 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 2-16 | F4 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-17 | F5 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 2-18 | F6 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 2-19 | F7 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 2-20 | F8 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-21 | F9 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 2-22 | F10 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 2-23 | F11 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 2-24 | F12 | 0.12 M | Monosaccharides | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-25 | G1 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 2-26 | G2 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 2-27 | G3 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 2-28 | G4 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-29 | G5 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 2-30 | G6 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 2-31 | G7 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 2-32 | G8 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-33 | G9 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 2-34 | G10 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 2-35 | G11 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 2-36 | G12 | 0.1 M | Carboxylic acids | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-37 | H1 | 0.1 M | Amino acids | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 1 |
| 2-38 | H2 | 0.1 M | Amino acids | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 2 |
| 2-39 | H3 | 0.1 M | Amino acids | 0.1 M | Buffer System 1 | 6.5 | 30 % v/v | Precipitant Mix 3 |
| 2-40 | H4 | 0.1 M | Amino acids | 0.1 M | Buffer System 1 | 6.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-41 | H5 | 0.1 M | Amino acids | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 1 |
| 2-42 | H6 | 0.1 M | Amino acids | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 2 |
| 2-43 | H7 | 0.1 M | Amino acids | 0.1 M | Buffer System 2 | 7.5 | 30 % v/v | Precipitant Mix 3 |
| 2-44 | H8 | 0.1 M | Amino acids | 0.1 M | Buffer System 2 | 7.5 | 37.5 % v/v | Precipitant Mix 4 |
| 2-45 | H9 | 0.1 M | Amino acids | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 1 |
| 2-46 | H10 | 0.1 M | Amino acids | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 2 |
| 2-47 | H11 | 0.1 M | Amino acids | 0.1 M | Buffer System 3 | 8.5 | 30 % v/v | Precipitant Mix 3 |
| 2-48 | H12 | 0.1 M | Amino acids | 0.1 M | Buffer System 3 | 8.5 | 37.5 % v/v | Precipitant Mix 4 |



Morpheus® II 10 mL, HT-96 and FX-96 pre-filled plate. MD1-91, MD1-92 and MD1-92-FX

Morpheus II is optimized to yield crystals not observed in traditional screens and also includes heavy atoms for experimental phasing as well as polyols for cryoprotection.

MD1-91 is presented as 96 x 10 mL conditions (*includes 12 x 15 mL glass bottles containing polyamine powders¹ to mix with water*). MD1-92 is presented as 96 x 1 mL conditions / MD1-92-FX is presented as 96 x 100 µL conditions.

Features of Morpheus II:

- Targeted incorporation of 35 low- molecular weight PDB ligands into 96 conditions.
- Incorporation of heavy atoms as additives for experimental phasing.
- Suitable for membrane proteins with PEGS and polyols as main precipitants.
- The inclusion of NDSBs, polyamines, amino acids and monosaccharides to enhance solubility and stability of many proteins for crystallization.
- New polyols included for cryoprotection of conditions: no need to investigate more conditions for cryo-cooling.
- Innovative buffer systems facilitate pH optimization.
- Readily available Morpheus® II Optimization reagents including the Mixes and Stock reagents.

Introduction

Morpheus II is a follow up to the original screen that was used with success in many laboratories. **Morpheus II** integrates reagents that are not seen in other screens, especially less common additives. As a consequence, the screen should have an impact not only on crystallisation but also on the overall structure determination process.

Morpheus II follows the general design principles of the original Morpheus screen. However, less typical additives have been included, such as heavy metals, NDSBs, polyamines, amino acids and monosaccharides. In addition, various polyols have been added for cryoprotection along with innovative buffer systems.

Some additives such as metal divalent cations can induce new crystal contacts. Also, heavy atoms were integrated to help with *ab initio* structure determination since a common issue to solve novel structure is the phase problem.

Most of the other additives are meant to alter protein stability and solubility (carboxylic acids, polyamines and monosaccharides).



Figure 1

Figure 1. Examples of crystals obtained during initial tests with the final formulation of Morpheus II. **A. B.** Concanavalin-A (MW = 27 kDa). **C.** Catalase (63 kDa). **D.** BAR domain (29 kDa). **E.** Trans-membrane complex (540 kDa). **F.** Actin homologue (37 kDa). **G.** Domain of a cytosolic nucleic acid sensor (12 kDa). **H.** Virus capsid (25 kDa). **I.** Polymerase complex (80 kDa).

¹ The mix of polyamines must be prepared and added to the 12 corresponding tubes by the user, hence only the raw chemicals are present in the screen kit (powder mix is given in a 15 ml glass bottle in order to prepare 13 ml using ultrapure water).



Screen Design

Morpheus II is based on the 3D grid design of Morpheus. The 35 PDB-derived ligands selected to formulate Morpheus2 are shown in Table 1. From these, eight additive mixes were prepared (Table 2): LiNaK, Divalents II, Alkalis, Oxometaltes, Lanthanides, Monosaccharides II, Amino acids II and Polyamines.

Formulation Notes

Morpheus II reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 μm filters. No preservatives are added.

PLEASE NOTE

The polyamine mix must be prepared and added to the 12 corresponding tubes by the user. The raw chemicals in a powder mix are provided in the kit in a 15 mL glass bottle. Sufficient ultrapure (>18.0 MΩ) water should be added to this bottle to make up a 13 mL solution. A light pellet may form in tubes 2-1 to 2-12/well numbers B-1 to B-12 which contain Lanthanides (2-1 to 2-12). It is easily re-suspended with gentle mixing.

The screen should be kept between 10-18 °C and gently mixed before use.

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

References

1. Gorrec, F (2009), The MORPHEUS protein crystallization screen *J Appl Cryst* **42**, 1035-1042
2. Gorrec, F (2013), The current approach to initial crystallization screening of proteins is under-sampled *J Appl Cryst* **46**, 795-797.

Morpheus II Optimization

Although the screen is composed of various mixes, consider each condition as for any other screen, with three stock solutions:

- mix of precipitants
- mix of additives
- mix of buffers.

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

Every condition can be made following the same ratio of stock solutions:

1/2 [Precipitant mix] + 1/10 [additive mix] + 1/10 [Buffer system] + 3/10 dH₂O.

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 II you can eventually investigate further, trying to reveal specificity of a single chemical.

For example, what happens when you replace the group of chemicals from a stock with only one chemical of this mix? (e.g. only one divalent cations instead of the corresponding mix of additives).

At this stage you may (or not) have a simpler condition to work with. You can also proceed to other "classic" optimization approaches such as using an additive screen, scale-up or seeding.



Table 1: List of PDB ligands in Morpheus II

| PDB ligand name | Class | PDB ID (main) | No. of structures* |
|--------------------------------------|-----------------|---------------|--------------------|
| Lithium sulfate | Common salt | LI | 51 |
| Sodium chloride | Common salt | NA | 4726 |
| Potassium sulfate | Common salt | K | 1638 |
| Manganese chloride tetrahydrate | Divalent cation | MN | 1938 |
| Cobalt chloride hexahydrate | Divalent cation | CO | 474 |
| Nickel chloride hexahydrate | Divalent cation | NI | 699 |
| Zinc acetate dihydrate | Divalent cation | ZN | 8413 |
| Barium acetate | Alkali | BA | 91 |
| Cesium acetate | Alkali | CS | 75 |
| Rubidium chloride | Alkali | RB | 34 |
| Strontium acetate | Alkali | SR | 101 |
| Sodium chromate tetrahydrate | Oxometalate | CR | 7 |
| Sodium molybdate dihydrate | Oxometalate | MOO | 20 |
| Sodium orthovanadate | Oxometalate | VO4 | 73 |
| Sodium tungstate dihydrate | Oxometalate | WO4 | 47 |
| Erbium (III) chloride hexahydrate | Lanthanide | ER3 | 2 |
| Terbium (III) chloride hexahydrate | Lanthanide | TB | 11 |
| Ytterbium (III) chloride hexahydrate | Lanthanide | YB | 57 |
| Yttrium (III) chloride hexahydrate | Lanthanide | YT3 | 33 |
| Xylitol | Monosaccharide | XYL | 25 |
| D-(-)-fructose | Monosaccharide | FRU; FUD | 36; 4 |
| D-sorbitol | Monosaccharide | SOR | 12 |
| Myo-inositol | Monosaccharide | INS | 16 |
| L-rhamnose monohydrate | Monosaccharide | RAM | 43 |
| DL-threonine | Amino-acid | DTH; THR | 23; n/a |
| DL-histidine, HCl, H2O | Amino-acid | DHI; HIS | 24; n/a |
| DL-5-hydroxylysine, HCl | Amino-acid | n/a; LYZ | 0; 7 |
| Trans-4-hydroxy-L-proline | Amino-acid | HYP | 149 |
| Spermine, 4HCl | Polyamine | SPM | 103 |
| Spermidine, 3HCl | Polyamine | SPD | 32 |
| 1,4-diaminobutane, 2HCl | Polyamine | PUT | 22 |
| DL-ornithine, HCl | Polyamine | ORD; ORN | 3; 56 |
| NDSB 256 | Surfactant | DMX | 4 |
| NDSB 195 | Surfactant | NDS | 7 |
| Bis-tris | Buffer | BTB | 114 |

*No of structures as determined by a query of the pdb carried out in December 2014



Table 2: Mixes of additives used in Morpheus II

| Mix name | Composition | Catalogue Number (100 ml) | Catalogue Number (250 ml) |
|--|---|---------------------------|---------------------------|
| 0.9 M LiNaK | 0.3 M Lithium sulfate, 0.3 M Sodium sulfate, 0.3 M Potassium sulfate | MD2-100-231 | MD2-250-231 |
| 0.02M Divalents II | 0.005M Manganese(II) chloride tetrahydrate, 0.005M Cobalt(II) chloride hexahydrate, 0.005M Nickel(II) chloride hexahydrate, 0.005M Zinc acetate dihydrate | MD2-100-232 | MD2-250-232 |
| 0.04 M Alkalis | 0.01M Rubidium chloride, 0.01M Strontium acetate, 0.01M Cesium acetate, 0.01M Barium acetate | MD2-100-233 | MD2-250-233 |
| 0.02 M Oxometalates | 0.005M Sodium chromate tetrahydrate, 0.005M Sodium molybdate dihydrate, 0.005M Sodium tungstate dihydrate, 0.005M Sodium orthovanadate | MD2-100-234 | MD2-250-234 |
| 0.02M Lanthanides | 0.005M Yttrium(III) chloride hexahydrate, 0.005M Erbium(III) chloride hexahydrate, 0.005M Terbium(III) chloride hexahydrate, 0.005M Ytterbium(III) chloride hexahydrate | MD2-100-235 | MD2-250-235 |
| 1M Monosaccharides II | 0.2M Xylitol, 0.2M <i>Myo</i> -Inositol, 0.2M D-(-)-Fructose, 0.2M L-Rhamnose monohydrate, 0.2M D-Sorbitol | MD2-100-236 | MD2-250-236 |
| 1M Amino acids II | 0.2M DL-Arginine hydrochloride, 0.2M DL-Threonine, 0.2M DL-Histidine monohydrochloride monohydrate, 0.2M DL-5-Hydroxylysine hydrochloride, 0.2M <i>trans</i> -4-hydroxy-L-proline | MD2-100-237 | MD2-250-237 |
| 0.4 M Polyamines (provided as powder for 10mL kits)* | 0.1M Spermine tetrahydrochloride, 0.1M Spermidine trihydrochloride, 0.1M 1,4-Diaminobutane dihydrochloride, 0.1M DL-Ornithine monohydrochloride | MD2-100-238 | MD2-250-238 |

Table 3: Buffer systems used in Morpheus II

| Mix name* | Conc. | pH @ 20°C | Composition | Catalogue Number (100 ml) | Catalogue Number (250 ml) |
|-----------------|-------|-----------|----------------------------|---------------------------|---------------------------|
| Buffer System 4 | 1.0M | 6.5 | MOPSO, Bis-Tris | MD2-100-243 | MD2-250-243 |
| Buffer System 5 | 1.0M | 7.5 | BES, Triethanolamine (TEA) | MD2-100-244 | MD2-250-244 |
| Buffer System 6 | 1.0M | 8.5 | Gly-Gly, AMPD | MD2-100-245 | MD2-250-245 |

*Buffer systems 1, 2 & 3 are allocated to the original Morpheus screen.

Table 4: Mixes of Precipitants used in Morpheus II

| Mix name* | Composition | Catalogue Number (100 ml) | Catalogue Number (250 ml) |
|-----------------------|--|---------------------------|---------------------------|
| 72% Precipitant Mix 5 | 30% w/v PEG 3000, 40% v/v 1, 2, 4-Butanetriol, 2% w/v NDSB 256 | MD2-100-239 | MD2-250-239 |
| 65% Precipitant Mix 6 | 25% w/v PEG 4000, 40% w/v 1,2,6-Hexanetriol | MD2-100-240 | MD2-250-240 |
| 60% Precipitant Mix 7 | 20% w/v PEG 8000, 40% v/v 1,5-Pentanediol | MD2-100-241 | MD2-250-241 |
| 62% Precipitant Mix 8 | 10% w/v PEG 20000, 50% w/v Trimethylpropane, 2% w/v NDSB 195 | MD2-100-242 | MD2-250-242 |

*precipitant Mixes 1, 2, 3 & 4 are allocated to the original Morpheus screen.



RE-ORDERING
INFORMATION

| Code | Pack Size | Description |
|---|-------------|---|
| MD1-91 | 96 x 10 mL | Morpheus II |
| MD1-92 | 96 x 1 mL | Morpheus II HT-96 |
| MD1-92-FX | 96x 100 µL | Morpheus II FX-96 pre-filled plate |
| Other Morpheus screens | | |
| MD1-46 | 96 x 10 mL | Morpheus |
| MD1-47 | 96 x 1 mL | Morpheus HT-96 |
| MD1-47-FX | 96x 100 µL | Morpheus FX-96 pre-filled plate |
| MD1-93 | 48 x 100 µL | The Morpheus® Additive screen |
| MD1-116 | 96 x 10 mL | Morpheus III |
| MD1-117 | 96 x 1 mL | Morpheus III HT-96 |
| MD1-118 | 48 x 100 µL | Hippocrates™ additive screen |
| Green screens (contain green fluorescent dye – ideal for UV) | | |
| MD1-46-GREEN | 96 x 10 mL | Morpheus Green screen |
| MD1-47-GREEN | 96 x 1 mL | Morpheus HT-96 Green screen |
| Combo Packs | | |
| MD1-76 | 192 x 10 mL | Power combo value pack (Morpheus + MIDASplus) |
| MD1-76-HT | 192 x 1 mL | Power combo value pack HT-96 (Morpheus + MIDASplus HT-96) |
| Single reagents | | |
| MDSR-91-tube number | 100 mL | Morpheus II single reagents |
| MDSR-92-well number | 100 mL | Morpheus II HT-96 single reagents |



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Morpheus II 10 mL
Morpheus II HT-96
Morpheus II FX-96

MD1-91
MD1-92
MD1-92-FX

Conditions 1-48 (Box 1)
Conditions A1-D12

| Screen ID | Well # | Conc. | Additives (PDB ligands) | Conc. | Buffer | pH | Conc. | Precipitant |
|-----------|--------|-------|-------------------------|-------|-----------------|-----|------------|-------------------|
| 1-1 | A1 | 90 mM | LiNaK | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 1-2 | A2 | 90 mM | LiNaK | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-3 | A3 | 90 mM | LiNaK | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 1-4 | A4 | 90 mM | LiNaK | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 1-5 | A5 | 90 mM | LiNaK | 0.1 M | Buffer System 5 | 7.5 | 36 % v/v | Precipitant Mix 5 |
| 1-6 | A6 | 90 mM | LiNaK | 0.1 M | Buffer System 5 | 7.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-7 | A7 | 90 mM | LiNaK | 0.1 M | Buffer System 5 | 7.5 | 30 % v/v | Precipitant Mix 7 |
| 1-8 | A8 | 90 mM | LiNaK | 0.1 M | Buffer System 5 | 7.5 | 31 % v/v | Precipitant Mix 8 |
| 1-9 | A9 | 90 mM | LiNaK | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 1-10 | A10 | 90 mM | LiNaK | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-11 | A11 | 90 mM | LiNaK | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 1-12 | A12 | 90 mM | LiNaK | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |
| 1-13 | B1 | 2 mM | Divalentes II | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 1-14 | B2 | 2 mM | Divalentes II | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-15 | B3 | 2 mM | Divalentes II | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 1-16 | B4 | 2 mM | Divalentes II | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 1-17 | B5 | 2 mM | Divalentes II | | none | | 36 % v/v | Precipitant Mix 5 |
| 1-18 | B6 | 2 mM | Divalentes II | | none | | 32.5 % v/v | Precipitant Mix 6 |
| 1-19 | B7 | 2 mM | Divalentes II | | none | | 30 % v/v | Precipitant Mix 7 |
| 1-20 | B8 | 2 mM | Divalentes II | | none | | 31 % v/v | Precipitant Mix 8 |
| 1-21 | B9 | 2 mM | Divalentes II | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 1-22 | B10 | 2 mM | Divalentes II | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-23 | B11 | 2 mM | Divalentes II | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 1-24 | B12 | 2 mM | Divalentes II | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |
| 1-25 | C1 | 4 mM | Alkalis | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 1-26 | C2 | 4 mM | Alkalis | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-27 | C3 | 4 mM | Alkalis | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 1-28 | C4 | 4 mM | Alkalis | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 1-29 | C5 | 4 mM | Alkalis | 0.1 M | Buffer System 5 | 7.5 | 36 % v/v | Precipitant Mix 5 |
| 1-30 | C6 | 4 mM | Alkalis | 0.1 M | Buffer System 5 | 7.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-31 | C7 | 4 mM | Alkalis | 0.1 M | Buffer System 5 | 7.5 | 30 % v/v | Precipitant Mix 7 |
| 1-32 | C8 | 4 mM | Alkalis | 0.1 M | Buffer System 5 | 7.5 | 31 % v/v | Precipitant Mix 8 |
| 1-33 | C9 | 4 mM | Alkalis | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 1-34 | C10 | 4 mM | Alkalis | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-35 | C11 | 4 mM | Alkalis | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 1-36 | C12 | 4 mM | Alkalis | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |
| 1-37 | D1 | 2 mM | Oxometalates | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 1-38 | D2 | 2 mM | Oxometalates | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-39 | D3 | 2 mM | Oxometalates | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 1-40 | D4 | 2 mM | Oxometalates | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 1-41 | D5 | 2 mM | Oxometalates | 0.1 M | Buffer System 5 | 7.5 | 36 % v/v | Precipitant Mix 5 |
| 1-42 | D6 | 2 mM | Oxometalates | 0.1 M | Buffer System 5 | 7.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-43 | D7 | 2 mM | Oxometalates | 0.1 M | Buffer System 5 | 7.5 | 30 % v/v | Precipitant Mix 7 |
| 1-44 | D8 | 2 mM | Oxometalates | 0.1 M | Buffer System 5 | 7.5 | 31 % v/v | Precipitant Mix 8 |
| 1-45 | D9 | 2 mM | Oxometalates | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 1-46 | D10 | 2 mM | Oxometalates | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 1-47 | D11 | 2 mM | Oxometalates | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 1-48 | D12 | 2 mM | Oxometalates | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |

Screen should be stored between 10-18°C and gently mixed before use

Morpheus II 10 mL
Morpheus II HT-96
Morpheus II FX-96MD1-91
MD1-92
MD1-92-FXConditions 49-96 (Box 2)
Conditions E1-H12

| Screen ID | Well # | Conc. | Additives (PDB ligands) | Conc. | Buffer | pH | Conc. | Precipitant |
|-----------|--------|--------|-------------------------|-------|-----------------|-----|------------|-------------------|
| 2-1 | E1 | 2 mM | Lanthanides* | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 2-2 | E2 | 2 mM | Lanthanides* | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-3 | E3 | 2 mM | Lanthanides* | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 2-4 | E4 | 2 mM | Lanthanides* | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 2-5 | E5 | 2 mM | Lanthanides* | 0.1 M | Buffer System 5 | 7.5 | 36 % v/v | Precipitant Mix 5 |
| 2-6 | E6 | 2 mM | Lanthanides* | 0.1 M | Buffer System 5 | 7.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-7 | E7 | 2 mM | Lanthanides* | 0.1 M | Buffer System 5 | 7.5 | 30 % v/v | Precipitant Mix 7 |
| 2-8 | E8 | 2 mM | Lanthanides* | 0.1 M | Buffer System 5 | 7.5 | 31 % v/v | Precipitant Mix 8 |
| 2-9 | E9 | 2 mM | Lanthanides* | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 2-10 | E10 | 2 mM | Lanthanides* | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-11 | E11 | 2 mM | Lanthanides* | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 2-12 | E12 | 2 mM | Lanthanides* | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |
| 2-13 | F1 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 2-14 | F2 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-15 | F3 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 2-16 | F4 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 2-17 | F5 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 5 | 7.5 | 36 % v/v | Precipitant Mix 5 |
| 2-18 | F6 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 5 | 7.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-19 | F7 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 5 | 7.5 | 30 % v/v | Precipitant Mix 7 |
| 2-20 | F8 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 5 | 7.5 | 31 % v/v | Precipitant Mix 8 |
| 2-21 | F9 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 2-22 | F10 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-23 | F11 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 2-24 | F12 | 100 mM | Monosaccharides II | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |
| 2-25 | G1 | 100 mM | Amino acids II | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 2-26 | G2 | 100 mM | Amino acids II | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-27 | G3 | 100 mM | Amino acids II | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 2-28 | G4 | 100 mM | Amino acids II | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 2-29 | G5 | 100 mM | Amino acids II | 0.1 M | Buffer System 5 | 7.5 | 36 % v/v | Precipitant Mix 5 |
| 2-30 | G6 | 100 mM | Amino acids II | 0.1 M | Buffer System 5 | 7.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-31 | G7 | 100 mM | Amino acids II | 0.1 M | Buffer System 5 | 7.5 | 30 % v/v | Precipitant Mix 7 |
| 2-32 | G8 | 100 mM | Amino acids II | 0.1 M | Buffer System 5 | 7.5 | 31 % v/v | Precipitant Mix 8 |
| 2-33 | G9 | 100 mM | Amino acids II | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 2-34 | G10 | 100 mM | Amino acids II | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-35 | G11 | 100 mM | Amino acids II | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 2-36 | G12 | 100 mM | Amino acids II | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |
| 2-37 | H1 | 40 mM | Polyamines† | 0.1 M | Buffer System 4 | 6.5 | 36 % v/v | Precipitant Mix 5 |
| 2-38 | H2 | 40 mM | Polyamines† | 0.1 M | Buffer System 4 | 6.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-39 | H3 | 40 mM | Polyamines† | 0.1 M | Buffer System 4 | 6.5 | 30 % v/v | Precipitant Mix 7 |
| 2-40 | H4 | 40 mM | Polyamines† | 0.1 M | Buffer System 4 | 6.5 | 31 % v/v | Precipitant Mix 8 |
| 2-41 | H5 | 40 mM | Polyamines† | 0.1 M | Buffer System 5 | 7.5 | 36 % v/v | Precipitant Mix 5 |
| 2-42 | H6 | 40 mM | Polyamines† | 0.1 M | Buffer System 5 | 7.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-43 | H7 | 40 mM | Polyamines† | 0.1 M | Buffer System 5 | 7.5 | 30 % v/v | Precipitant Mix 7 |
| 2-44 | H8 | 40 mM | Polyamines† | 0.1 M | Buffer System 5 | 7.5 | 31 % v/v | Precipitant Mix 8 |
| 2-45 | H9 | 40 mM | Polyamines† | 0.1 M | Buffer System 6 | 8.5 | 36 % v/v | Precipitant Mix 5 |
| 2-46 | H10 | 40 mM | Polyamines† | 0.1 M | Buffer System 6 | 8.5 | 32.5 % v/v | Precipitant Mix 6 |
| 2-47 | H11 | 40 mM | Polyamines† | 0.1 M | Buffer System 6 | 8.5 | 30 % v/v | Precipitant Mix 7 |
| 2-48 | H12 | 40 mM | Polyamines† | 0.1 M | Buffer System 6 | 8.5 | 31 % v/v | Precipitant Mix 8 |

Screen should be stored between 10-18°C and gently mixed before use

*A light pellet may form in tubes 2-1 to 2-12/well numbers B-1 to B-12 which contain Lanthanides (2-1 to 2-12). It is easily re-suspended with gentle mixing.

†The polyamine mix must be prepared and added to the 12 corresponding tubes by the user. The raw chemicals in a powder mix are provided in the kit in a 15 mL glass bottle. Sufficient ultrapure (>18.0 MΩ) water should be added to this bottle to make up a 13 mL solution.



Morpheus® III 10 mL and HT-96 MD1-116 and MD1-117

Morpheus III uniquely contains a range of small, drug-like compounds to aid protein stabilisation and crystallisation.

MD1-116 is presented as 96 x 10 mL conditions. MD1-117 is presented as 96 x 1 mL conditions.

Let the unique drug-like additives in Morpheus III help cure your crystallisation problems:

- Expands the amount of chemical space screened with unique drug-like additives.
- Drug-like compounds can aid protein-stabilisation and are often found in structures in the PDB.
- Hippocrates additive screen contains all 44 compounds used in Morpheus III for easy optimization.
- Designed *de novo* and optimised against a broad range of protein samples.
- No bias to particular reagents or macromolecules.
- Developed by Dr Fabrice Gorrec of the MRC-LMB, Cambridge, UK, the creator of a range of popular and novel screens including Morpheus and the LMB Crystallisation screen.

Introduction

Morpheus III is the latest member of the Morpheus® family of protein screens. It contains a range of drug-like compounds not present in other crystallisation screens. The additives are often found bound to protein structures submitted to the pdb and may therefore increase stability and thus crystallisability.

Morpheus III follows the general design principles of the original Morpheus screen. However, in this case a drug-like additives such as antibiotics, dipeptides and phytochemicals have been added. In addition, each condition has some cryoprotectant along with the innovative buffer systems seen with other Morpheus screens.

These drug-like compounds are likely to interact with proteins of primary interest to those in the pharmaceutical industry or researching the causes of human disease. As such they may improve the protein stability and solubility of many targets for macromolecular structure solution.

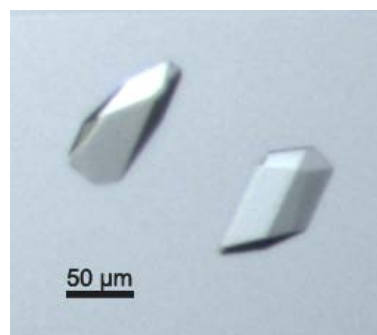


Figure 1. USB1 (2H phosphoesterase) crystals grown with Morpheus III. With thanks to Dr C Hilcenko, University of Cambridge).

Screen Design

Morpheus III is based on the 3D grid design of Morpheus (Figure 2). The drug-like ligands selected to formulate Morpheus III are shown in Table 1. From these, eight additive mixes were prepared (Table 2): Dipeptides, Vitamins, Nucleosides, Phytochemicals 1, Phytochemicals 2, Antibiotics, Cholic acid derivatives and Anaesthetic alkaloids.

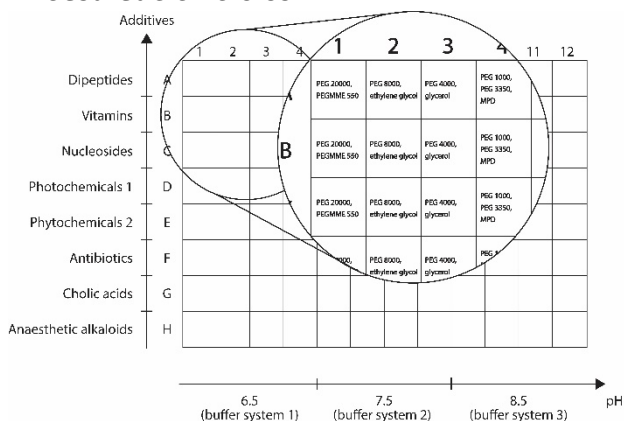


Figure 2: Morpheus III 3D grid design

**Formulation Notes:**

Morpheus III reagents, with the exception of the Phytochemicals 1 and Phytochemicals 2 mixes, are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 μm filters. No preservatives are added.

The Phytochemicals 1 (MD2-50-316 and MD2-100-316) and Phytochemicals 2 (MD2-50-317 and MD2-100-317) mixes are dissolved in 50% EtOH and 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, product details and manufacturer's datasheets can be found at www.moleculardimensions.com.

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

References

1. Gorrec, F (2009), The MORPHEUS protein crystallization screen *J Appl Cryst* **42**, 1035-1042
2. Gorrec, F (2013), The current approach to initial crystallization screening of proteins is under-sampled *J Appl Cryst* **46**, 795-797.
3. Gorrec, F (2015), The Morpheus II protein crystallization screen, ICCBM15 proceedings (Special Issue ActaF).

**RE-ORDERING
INFORMATION**

| Code | Pack Size | Description |
|---------|-------------|-------------------------------|
| MD1-116 | 96 x 10 mL | Morpheus III |
| MD1-117 | 96 x 1 mL | Morpheus III HT-96 |
| MD1-118 | 48 x 100 μL | Hippocrates™ additive screen |
| MD1-91 | 96 x 10 mL | Morpheus II |
| MD1-92 | 96 x 1 mL | Morpheus II HT-96 |
| MD1-46 | 96 x 10 mL | Morpheus |
| MD1-47 | 96 x 1 mL | Morpheus HT-96 |
| MD1-93 | 48 x 100 μL | The Morpheus® Additive screen |

Morpheus, Morpheus II and Morpheus III have been designed and developed by Fabrice GORREC, in collaboration with the scientists at the Medical Research Council Laboratory of Molecular Biology (LMB) at Cambridge and is manufactured exclusively under license from LifeARC by Molecular Dimensions Limited.

Limited Use and Restrictions: Products sold by Molecular Dimensions Ltd. or its affiliates or authorized distributors and information relating to same are intended for research use only in crystal growth and optimization of crystal growth following use of the product by the purchaser and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, including resale or use in manufacture. The license to use Morpheus and Morpheus II specifically excludes any rights to use the product information for the manufacture of the product or derivatives thereof, or distribute, transfer, or otherwise provide access to such information to any third party for any purpose or use.



Table 1: List of Drug-like ligands in Morpheus III

| Ligand Name | Mix | PDB ID | No of Structures [†] |
|--|-------------------------|--------|-------------------------------|
| Ala-Ala | Dipeptides | n/a | - |
| Ala-Gln | Dipeptides | n/a | 1 |
| Gly-Glu | Dipeptides | n/a | - |
| Gly-L-Ala | Dipeptides | n/a | - |
| Gly-L-Asp | Dipeptides | n/a | - |
| Gly-Sar | Dipeptides | n/a | - |
| L-Carnosine | Dipeptides | n/a | - |
| Leu-Ala hydrate | Dipeptides | n/a | 1 |
| Sodium-L-ascorbate | Vitamins* | ASC | 33 |
| Choline chloride | Vitamins* | CHT | 37 |
| D-Panthenol | Vitamins* | MV2 | 1 |
| Pyridoxine hydrochloride | Vitamins* | UEG | 3 |
| Thiamine hydrochloride | Vitamins* | VIB | 12 |
| Cytidine | Nucleosides | CTN | 23 |
| Inosine | Nucleosides | NOS | 14 |
| Ribavirin | Nucleosides | RBV | 3 |
| Thymidine | Nucleosides | THM | 45 |
| Uridine | Nucleosides | URI | 27 |
| Menthol | Phytochemicals 1* | n/a | - |
| Caffeic acid | Phytochemicals 1* | DHC | 9 |
| D-Quinic acid | Phytochemicals 1* | QIC | 3 |
| Shikimic acid | Phytochemicals 1* | SKM | 36 |
| Gallic acid monohydrate | Phytochemicals 1* | GDE | 9 |
| N-Vanillylnonanamide | Phytochemicals 1* | n/a | - |
| Thymol | Phytochemicals 1* | IPB | 1 |
| D-Salicin | Phytochemicals 2 | SAO | 1 |
| Esculin hydrate | Phytochemicals 2 | n/a | - |
| Quinine hemisulfate salt monohydrate | Phytochemicals 2 | QI9 | 3 |
| Tryptamine | Phytochemicals 2 | TSS | 7 |
| Arbutin | Phytochemicals 2 | n/a | - |
| Ampicillin sodium salt | Antibiotics | AIC | 8 |
| Apramycin sulfate salt | Antibiotics | AM2 | 7 |
| Bacitracin | Antibiotics | n/a | - |
| Dihydrostreptomycin sesquisulfate | Antibiotics | SRY | 19 |
| Gentamicin sulfate | Antibiotics | 51G | 3 |
| Spectinomycin dihydrochloride pentahydrate | Antibiotics | SCM | 3 |
| CHAPS | Cholic acid derivatives | CPS | 60 |
| CHAPSO | Cholic acid derivatives | 1N7 | 9 |
| Sodium glycocholate hydrate | Cholic acid derivatives | GCH | 4 |
| Taurocholic acid sodium salt hydrate | Cholic acid derivatives | TCH | 8 |
| Lidocaine hydrochloride | Anesthetic alkaloids | LQZ | 2 |
| Procaine hydrochloride | Anesthetic alkaloids | n/a | - |
| Proparacaine hydrochloride | Anesthetic alkaloids | n/a | - |
| Tetracaine hydrochloride | Anesthetic alkaloids | TE4 | 2 |

[†]No. of Structures requested in April 2018

*Please note that the Vitamin and Phytochemicals 1 mixes may darken with age.

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

| Mix name | Composition | Catalogue Number (50 ml) | Catalogue Number (100 ml) |
|--|---|--------------------------|---------------------------|
| 16% w/v Dipeptides | 2% w/v Ala-Ala, 2% w/v Ala-Gln, 2% w/v Gly-Glu, 2% w/v Gly-L-Ala, 2% w/v Gly-L-Asp, 2% w/v Gly-Sar, 2% w/v L-Carnosine, 2% w/v Leu-Ala hydrate | MD2-50-313 | MD2-100-313 |
| 15% w/v Vitamins* | 3% w/v Sodium-L ascorbate, 3% w/v Choline Chloride, 3% v/v D-Panthenol, 3% w/v Pyridoxine hydrochloride, 3% w/v Thiamine hydrochloride | MD2-50-314 | MD2-100-314 |
| 10% w/v Nucleosides | 2% w/v Cytidine, 2% w/v Inosine, 2% w/v Ribavirin, 2% w/v Thymidine, 2% w/v Uridine | MD2-50-315 | MD2-100-315 |
| 3.5% w/v Phytochemicals 1 [†] | 0.5% w/v (-)-Menthol, 0.5% w/v Caffeic acid, 0.5% w/v D-Quinic acid, 0.5% w/v Shikimic acid, 0.5% w/v Gallic acid monohydrate, 0.5% w/v N-Vanillynonanamide, 0.5% w/v Thymol | MD2-50-316 | MD2-100-316 |
| 2.5% w/v Phytochemicals 2 [†] | 0.5% w/v D-Salicin, 0.5% w/v Esculin hydrate, 0.5% w/v Quinine hemisulfate salt monohydrate, 0.5% w/v Tryptamine, 0.5% w/v Arbutin | MD2-50-317 | MD2-100-317 |
| 6% w/v Antibiotics | 1% w/v Ampicillin sodium salt, 1% w/v Apramycin sulfate salt, 1% w/v Bacitracin, 1% w/v Dihydrostreptomycin sesquisulfate, 1% w/v Gentamicin sulfate, 1% w/v Spectinomycin dihydrochloride pentahydrate | MD2-50-318 | MD2-100-318 |
| 12% w/v Cholic acid derivatives | 3% w/v CHAPS, 3% w/v CHAPSO, 3% w/v Sodium glycocholate hydrate, 3% w/v Taurocholic acid sodium salt hydrate | MD2-50-319 | MD2-100-319 |
| 8% w/v Anesthetic alkaloids | 2% w/v Lidocaine hydrochloride monohydrate, 2% w/v Procaine hydrochloride, 2% w/v Proparacaine hydrochloride, 2% w/v tetracaine hydrochloride | MD2-50-320 | MD2-100-320 |

[†]3.5% Phytochemicals 1 (MD2-50-316 and MD2-100-316) and 2.5% Phytochemicals 2 (MD2-50-317 and MD2-100-317) are dissolved in 50% EtOH.

*Please note that the Vitamin and Phytochemicals 1 mixes may darken with age.

Table 3: Buffer systems used in Morpheus III

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



Table 4: Mixes of Precipitants used in Morpheus III

| Mix name | Composition | Catalogue Number (100 mL) | Catalogue Number (250 mL) |
|-----------------------|---|---------------------------|---------------------------|
| 60% Precipitant Mix 1 | 40% v/v PEG 500* MME; 20 % w/v PEG 20000 | MD2-100-81 | MD2-250-81 |
| 60% Precipitant Mix 2 | 40% v/v Ethylene glycol; 20 % w/v PEG 8000 | MD2-100-82 | MD2-250-82 |
| 60% Precipitant Mix 3 | 40% v/v Glycerol; 20% w/v PEG 4000 | MD2-100-83 | MD2-250-83 |
| 75% Precipitant Mix 4 | 25% v/v MPD; 25% PEG 1000; 25% w/v PEG 3350 | MD2-100-84 | MD2-250-84 |

Morpheus III Optimization

Although the screen is composed of various mixes, consider each condition as for any other screen, with three stock solutions:

- mix of precipitants
- mix of additives
- mix of buffers.

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

Every condition can be made following the same ratio of stock solutions:

1/2 [Precipitant mix] + 1/10 [additive mix] + 1/10 [Buffer system] + 3/10 dH₂O.

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 III you can eventually investigate further, trying to reveal specificity of a single chemical.

For example, what happens when you replace the group of chemicals from a stock with only one chemical of this mix? (e.g. only one divalent cations instead of the corresponding mix of additives).

At this stage you may (or not) have a simpler condition to work with. You can also proceed to other "classic" optimization approaches such as using an additive screen, scale-up or seeding.

Morpheus III MD1-116 (Box 1)
Morpheus III HT-96 MD1-117Conditions 1-48
Conditions A1-D12

| Tube # | Well # | Conc. | Ligand | Conc. | Buffer | pH | Conc. | Precipitant |
|--------|--------|--------|------------------------|-------|-----------------|-----|--------|-------------------|
| 1-1 | A1 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 1-2 | A2 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 1-3 | A3 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 1-4 | A4 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 1-5 | A5 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 1-6 | A6 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 1-7 | A7 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 1-8 | A8 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 1-9 | A9 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 1-10 | A10 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 1-11 | A11 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 1-12 | A12 | 1.6 % | Dipeptides Mix | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |
| 1-13 | B1 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 1-14 | B2 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 1-15 | B3 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 1-16 | B4 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 1-17 | B5 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 1-18 | B6 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 1-19 | B7 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 1-20 | B8 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 1-21 | B9 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 1-22 | B10 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 1-23 | B11 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 1-24 | B12 | 1.5 % | Vitamins mix* | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |
| 1-25 | C1 | 1 % | Nucleosides mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 1-26 | C2 | 1 % | Nucleosides mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 1-27 | C3 | 1 % | Nucleosides mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 1-28 | C4 | 1 % | Nucleosides mix | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 1-29 | C5 | 1 % | Nucleosides mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 1-30 | C6 | 1 % | Nucleosides mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 1-31 | C7 | 1 % | Nucleosides mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 1-32 | C8 | 1 % | Nucleosides mix | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 1-33 | C9 | 1 % | Nucleosides mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 1-34 | C10 | 1 % | Nucleosides mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 1-35 | C11 | 1 % | Nucleosides mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 1-36 | C12 | 1 % | Nucleosides mix | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |
| 1-37 | D1 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 1-38 | D2 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 1-39 | D3 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 1-40 | D4 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 1-41 | D5 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 1-42 | D6 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 1-43 | D7 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 1-44 | D8 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 1-45 | D9 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 1-46 | D10 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 1-47 | D11 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 1-48 | D12 | 0.35 % | Phytochemicals 1 mix†* | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |

†The Phytochemicals 1 and Phytochemicals 2 mixes are dissolved in 50% EtOH.

*Please note that the Vitamin and Phytochemicals mixes may darken with age.



| Tube # | Well # | Conc. | Ligand | Conc. | Buffer | pH | Conc. | Precipitant |
|--------|--------|--------|-----------------------------|-------|-----------------|-----|--------|-------------------|
| 2-1 | E1 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 2-2 | E2 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 2-3 | E3 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 2-4 | E4 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 2-5 | E5 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 2-6 | E6 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 2-7 | E7 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 2-8 | E8 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 2-9 | E9 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 2-10 | E10 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 2-11 | E11 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 2-12 | E12 | 0.25 % | Phytochemicals 2 mix† | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |
| 2-13 | F1 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 2-14 | F2 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 2-15 | F3 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 2-16 | F4 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 2-17 | F5 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 2-18 | F6 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 2-19 | F7 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 2-20 | F8 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 2-21 | F9 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 2-22 | F10 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 2-23 | F11 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 2-24 | F12 | 0.6 % | Antibiotics mix | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |
| 2-25 | G1 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 2-26 | G2 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 2-27 | G3 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 2-28 | G4 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 2-29 | G5 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 2-30 | G6 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 2-31 | G7 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 2-32 | G8 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 2-33 | G9 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 2-34 | G10 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 2-35 | G11 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 2-36 | G12 | 1.2 % | Cholic acid derivatives mix | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |
| 2-37 | H1 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 1 |
| 2-38 | H2 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 2 |
| 2-39 | H3 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 1 | 6.5 | 30 % | Precipitant Mix 3 |
| 2-40 | H4 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 1 | 6.5 | 37.5 % | Precipitant Mix 4 |
| 2-41 | H5 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 1 |
| 2-42 | H6 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 2 |
| 2-43 | H7 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 2 | 7.5 | 30 % | Precipitant Mix 3 |
| 2-44 | H8 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 2 | 7.5 | 37.5 % | Precipitant Mix 4 |
| 2-45 | H9 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 1 |
| 2-46 | H10 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 2 |
| 2-47 | H11 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 3 | 8.5 | 30 % | Precipitant Mix 3 |
| 2-48 | H12 | 0.8 % | Anesthetic alkaloids mix | 0.1 M | Buffer System 3 | 8.5 | 37.5 % | Precipitant Mix 4 |

†The Phytochemicals 1 and Phytochemicals 2 mixes are dissolved in 50% EtOH.