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.

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.

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.

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

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

<table>
<thead>
<tr>
<th>Code</th>
<th>Pack Size</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>MD1-116</td>
<td>96 x 10 mL</td>
<td>Morpheus III</td>
</tr>
<tr>
<td>MD1-117</td>
<td>96 x 1 mL</td>
<td>Morpheus III HT-96</td>
</tr>
<tr>
<td>MD1-118</td>
<td>48 x 100 µL</td>
<td>Hippocrates™ II additive screen</td>
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<td>MD1-91</td>
<td>96 x 10 mL</td>
<td>Morpheus II</td>
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<td>MD1-92</td>
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<td>Morpheus II HT-96</td>
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<td>MD1-46</td>
<td>96 x 10 mL</td>
<td>Morpheus</td>
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<td>MD1-47</td>
<td>96 x 1 mL</td>
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<td>MD1-93</td>
<td>48 x 100 µL</td>
<td>The Morpheus® Additive screen</td>
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**Table 1: List of Drug-like ligands in Morpheus III**

<table>
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<tr>
<th>Ligand Name</th>
<th>Mix</th>
<th>PDB ID</th>
<th>No of Structures†</th>
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<td>Ala-Ala</td>
<td>Dipeptides</td>
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<td>Ala-Gln</td>
<td>Dipeptides</td>
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<td>Dipeptides</td>
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<td>Gly-Sar</td>
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<td>Sodium-L-ascorbate</td>
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<td>Vitamins*</td>
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<td>Nucleosides</td>
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<td>Thymol</td>
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<td>Quinine hemisulfate salt monohydrate</td>
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<td>TSS</td>
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<td>Arbutin</td>
<td>Phytochemicals 2</td>
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<td>Antibiotics</td>
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<td>Apramycin sulfate salt</td>
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<td>Dihydrostreptomycin sesquisulfate</td>
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<td>SRY</td>
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<td>Spectinomycin dihydrochloride pentahydrate</td>
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<td>SCM</td>
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<td>Cholic acid derivatives</td>
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<td>Procaine hydrochloride</td>
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<td>Tetracaine hydrochloride</td>
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†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

<table>
<thead>
<tr>
<th>Mix name</th>
<th>Composition</th>
<th>Catalogue Number (50 ml)</th>
<th>Catalogue Number (100 ml)</th>
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<tbody>
<tr>
<td>16% w/v Dipeptides</td>
<td>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</td>
<td>MD2-50-313</td>
<td>MD2-100-313</td>
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<tr>
<td>15% w/v Vitamins*</td>
<td>3% w/v Sodium-L ascorbate, 3% w/v Choline Chloride, 3% w/v D-Panthenol, 3% w/v Pyridoxine hydrochloride, 3% w/v Thiamine hydrochloride</td>
<td>MD2-50-314</td>
<td>MD2-100-314</td>
</tr>
<tr>
<td>10% w/v Nucleosides</td>
<td>2% w/v Cytidine, 2% w/v Inosine, 2% w/v Ribavirin, 2% w/v Thymidine, 2% w/v Uridine</td>
<td>MD2-50-315</td>
<td>MD2-100-315</td>
</tr>
<tr>
<td>3.5% w/v Phytochemicals 1†</td>
<td>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-Vanillylnonanamide, 0.5% w/v Thymol</td>
<td>MD2-50-316</td>
<td>MD2-100-316</td>
</tr>
<tr>
<td>2.5% w/v Phytochemicals 2†</td>
<td>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</td>
<td>MD2-50-317</td>
<td>MD2-100-317</td>
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<tr>
<td>6% w/v Antibiotics</td>
<td>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</td>
<td>MD2-50-318</td>
<td>MD2-100-318</td>
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<tr>
<td>12% w/v Cholic acid derivatives</td>
<td>3% w/v CHAPS, 3% w/v CHAPSO, 3% w/v Sodium glycocholate hydrate, 3% w/v Taurocholic acid sodium salt hydrate</td>
<td>MD2-50-319</td>
<td>MD2-100-319</td>
</tr>
<tr>
<td>8% w/v Anesthetic alkaloids</td>
<td>2% w/v Lidocaine hydrochloride monohydrate, 2% w/v Procaine hydrochloride, 2% w/v Proparacaine hydrochloride, 2% w/v tetracaine hydrochloride</td>
<td>MD2-50-320</td>
<td>MD2-100-320</td>
</tr>
</tbody>
</table>

†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

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<tr>
<th>Mix name</th>
<th>Conc.</th>
<th>pH @ 20°C</th>
<th>Composition</th>
<th>Catalogue Number (100 mL)</th>
<th>Catalogue Number (250 mL)</th>
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</thead>
<tbody>
<tr>
<td>Buffer System 1</td>
<td>1.0M</td>
<td>6.5</td>
<td>Imidazole; MES monohydrate (acid)</td>
<td>MD2-100-100</td>
<td>MD2-250-100</td>
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<tr>
<td>Buffer System 2</td>
<td>1.0M</td>
<td>7.5</td>
<td>Sodium HEPES; MOPS (acid)</td>
<td>MD2-100-101</td>
<td>MD2-250-101</td>
</tr>
<tr>
<td>Buffer System 3</td>
<td>1.0M</td>
<td>8.5</td>
<td>Tris (base); BICINE</td>
<td>MD2-100-102</td>
<td>MD2-250-102</td>
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</table>
Table 4: Mixes of Precipitants used in Morpheus III

<table>
<thead>
<tr>
<th>Mix name</th>
<th>Composition</th>
<th>Catalogue Number (100 mL)</th>
<th>Catalogue Number (250 mL)</th>
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<tr>
<td>60% Precipitant Mix 1</td>
<td>40% v/v PEG 500° MME; 20% w/v PEG 20000</td>
<td>MD2-100-81</td>
<td>MD2-250-81</td>
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<tr>
<td>60% Precipitant Mix 2</td>
<td>40% v/v Ethylene glycol; 20% w/v PEG 8000</td>
<td>MD2-100-82</td>
<td>MD2-250-82</td>
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<tr>
<td>60% Precipitant Mix 3</td>
<td>40% v/v Glycerol; 20% w/v PEG 4000</td>
<td>MD2-100-83</td>
<td>MD2-250-83</td>
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<tr>
<td>75% Precipitant Mix 4</td>
<td>25% v/v MPD; 25% PEG 1000; 25% w/v PEG 3350</td>
<td>MD2-100-84</td>
<td>MD2-250-84</td>
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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:

\[
\frac{1}{2} \text{[Precipitant mix]} + \frac{1}{10} \text{[additive mix]} + \frac{1}{10} \text{[Buffer system]} + \frac{3}{10} \text{dH}_2\text{O}.
\]

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

*Please note that the vitamin and phytochemicals mixes may darken with age.

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Well #</th>
<th>Conc.</th>
<th>Ligand</th>
<th>Conc.</th>
<th>Buffer</th>
<th>pH</th>
<th>Conc.</th>
<th>Precipitant</th>
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<td>A1</td>
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<td>0.1 M Buffer System 1</td>
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<td>Dipeptides Mix</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
<td>30%</td>
<td>Precipitant Mix 2</td>
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<td>1-3</td>
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<td>1.6%</td>
<td>Dipeptides Mix</td>
<td>0.1 M Buffer System 1</td>
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<td>Precipitant Mix 3</td>
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<td>0.1 M Buffer System 1</td>
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<td>0.1 M Buffer System 3</td>
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<td>B4</td>
<td>1.5%</td>
<td>Vitamins mix*</td>
<td>0.1 M Buffer System 2</td>
<td>7.5</td>
<td>30%</td>
<td>Precipitant Mix 1</td>
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</tr>
<tr>
<td>1-17</td>
<td>B5</td>
<td>1.5%</td>
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<td>0.1 M Buffer System 2</td>
<td>7.5</td>
<td>30%</td>
<td>Precipitant Mix 2</td>
<td></td>
</tr>
<tr>
<td>1-18</td>
<td>B6</td>
<td>1.5%</td>
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<td>0.1 M Buffer System 2</td>
<td>7.5</td>
<td>30%</td>
<td>Precipitant Mix 3</td>
<td></td>
</tr>
<tr>
<td>1-19</td>
<td>B7</td>
<td>1.5%</td>
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<td>0.1 M Buffer System 2</td>
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<td>30%</td>
<td>Precipitant Mix 4</td>
<td></td>
</tr>
<tr>
<td>1-20</td>
<td>B8</td>
<td>1.5%</td>
<td>Vitamins mix*</td>
<td>0.1 M Buffer System 3</td>
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<td>30%</td>
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</tr>
<tr>
<td>1-21</td>
<td>B9</td>
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<td>8.5</td>
<td>30%</td>
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<td></td>
</tr>
<tr>
<td>1-22</td>
<td>B10</td>
<td>1.5%</td>
<td>Vitamins mix*</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>30%</td>
<td>Precipitant Mix 3</td>
<td></td>
</tr>
<tr>
<td>1-23</td>
<td>B11</td>
<td>1.5%</td>
<td>Vitamins mix*</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>30%</td>
<td>Precipitant Mix 4</td>
<td></td>
</tr>
<tr>
<td>1-24</td>
<td>B12</td>
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<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>37.5%</td>
<td>Precipitant Mix 1</td>
<td></td>
</tr>
<tr>
<td>1-25</td>
<td>C1</td>
<td>1%</td>
<td>Nucleosides mix</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
<td>30%</td>
<td>Precipitant Mix 1</td>
<td></td>
</tr>
<tr>
<td>1-26</td>
<td>C2</td>
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<td>Nucleosides mix</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
<td>30%</td>
<td>Precipitant Mix 2</td>
<td></td>
</tr>
<tr>
<td>1-27</td>
<td>C3</td>
<td>1%</td>
<td>Nucleosides mix</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
<td>37.5%</td>
<td>Precipitant Mix 3</td>
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</tr>
<tr>
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<td>C4</td>
<td>1%</td>
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<td>0.1 M Buffer System 2</td>
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<td>30%</td>
<td>Precipitant Mix 1</td>
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</tr>
<tr>
<td>1-29</td>
<td>C5</td>
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<td>Nucleosides mix</td>
<td>0.1 M Buffer System 2</td>
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<td>30%</td>
<td>Precipitant Mix 2</td>
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<td>0.1 M Buffer System 2</td>
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<td>37.5%</td>
<td>Precipitant Mix 4</td>
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</tr>
<tr>
<td>1-32</td>
<td>C8</td>
<td>1%</td>
<td>Nucleosides mix</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>30%</td>
<td>Precipitant Mix 1</td>
<td></td>
</tr>
<tr>
<td>1-33</td>
<td>C9</td>
<td>1%</td>
<td>Nucleosides mix</td>
<td>0.1 M Buffer System 3</td>
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<td>30%</td>
<td>Precipitant Mix 2</td>
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</tr>
<tr>
<td>1-34</td>
<td>C10</td>
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<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>30%</td>
<td>Precipitant Mix 3</td>
<td></td>
</tr>
<tr>
<td>1-35</td>
<td>C11</td>
<td>1%</td>
<td>Nucleosides mix</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>37.5%</td>
<td>Precipitant Mix 4</td>
<td></td>
</tr>
<tr>
<td>1-36</td>
<td>C12</td>
<td>1%</td>
<td>Nucleosides mix</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>37.5%</td>
<td>Precipitant Mix 1</td>
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</tr>
<tr>
<td>1-37</td>
<td>D1</td>
<td>0.35%</td>
<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
<td>30%</td>
<td>Precipitant Mix 1</td>
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<tr>
<td>1-38</td>
<td>D2</td>
<td>0.35%</td>
<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
<td>30%</td>
<td>Precipitant Mix 2</td>
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<tr>
<td>1-39</td>
<td>D3</td>
<td>0.35%</td>
<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
<td>30%</td>
<td>Precipitant Mix 3</td>
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<tr>
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<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 1</td>
<td>6.5</td>
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<td>1-41</td>
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<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 2</td>
<td>7.5</td>
<td>30%</td>
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<tr>
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<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 2</td>
<td>7.5</td>
<td>30%</td>
<td>Precipitant Mix 2</td>
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</tr>
<tr>
<td>1-43</td>
<td>D7</td>
<td>0.35%</td>
<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 2</td>
<td>7.5</td>
<td>30%</td>
<td>Precipitant Mix 3</td>
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</tr>
<tr>
<td>1-44</td>
<td>D8</td>
<td>0.35%</td>
<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 2</td>
<td>7.5</td>
<td>37.5%</td>
<td>Precipitant Mix 4</td>
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<tr>
<td>1-45</td>
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<td>0.35%</td>
<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>30%</td>
<td>Precipitant Mix 1</td>
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<tr>
<td>1-46</td>
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<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>30%</td>
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</tr>
<tr>
<td>1-47</td>
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<td>0.35%</td>
<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 3</td>
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<td>30%</td>
<td>Precipitant Mix 3</td>
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</tr>
<tr>
<td>1-48</td>
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<td>Phytochemicals 1 mix†</td>
<td>0.1 M Buffer System 3</td>
<td>8.5</td>
<td>37.5%</td>
<td>Precipitant Mix 4</td>
<td></td>
</tr>
</tbody>
</table>

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

*Please note that the vitamin and phytochemicals mixes may darken with age.
The Phytochemicals 1 and Phytochemicals 2 mixes are dissolved in 50% EtOH.