

Applications

Crystallization screen for proteins, peptides, nucleic acids and water soluble small molecules.

Features

Screens classic, contemporary and new crystallization reagent systems Compatible with Micro Batch, Vapor Diffusion and Liquid Diffusion methods. Samples pH 3.5 to 8.5. Specially formulated reagent zones include:

- Classic salts versus pH
- Neutralized organic acids
- High [salt] with low [polymer]
- High [polymer] with low [salt]
- Low ionic strength versus pH
- PEG & Salt versus pH
- PEG & Salt

General Description

Index HT™ is supplied in a sterile, polypropylene 96 Deep Well block, each reservoir containing 1 ml of sterile filtered reagent. The block is heat sealed using a special polypropylene backed film.

Index is kit of 96 preformulated reagents designed to provide a rapid screening method for the crystallization of biological macromolecules including proteins, peptides, and nucleic acids. Index is a straightforward, effective, and practical kit for determining preliminary crystallization conditions. Index is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

Background

Index is designed as a 96 reagent crystallization screen that combines the strategies of the Grid Screen, Sparse Matrix, and Incomplete Factorial with classical, contemporary and new crystallization reagent systems into a highly effective and efficient format. Index was designed, developed and evaluated to: 1) be compatible with Micro Batch, Vapor Diffusion, and Liquid Diffusion crystallization methodologies, 2) evaluate classical, contemporary and new crystallization reagent systems, 3) efficiently sample crystallization reagent, concentration and pH space using 96 conditions, 4) combine the most effective features of the Grid Screen, Sparse Matrix and Incomplete Factorial methodologies, and 5) demonstrate that each condition is effective as producing crystals of biological macromolecules.

Index crystallization reagents are compatible with Paraffin (mineral) and Silicon based crystallization oils. Index is the first commercially available crystallization screen specifically designed and available to be compatible with Micro Batch, Vapor or Liquid Diffusion crystallization methodologies.

Index utilizes a broad, yet selective portfolio of crystallization reagent systems which encompasses the following: Classic reagents such as Ammo-

onium sulfate and Sodium potassium phosphate. Contemporary reagents such as Polyethylene glycols and MPD. New crystallization reagent systems such as the neutralized Organic Acids Sodium malonate and Succinate along with Tacsimate and the Pentaerythritols. These reagent systems are formulated across a sparse matrix and incomplete factorial of concentration ranges, a pH range of 3.5 to 8.5, low ionic strength, high ionic strength, and mixed polymer/salt including halides for potential phasing.

Index samples the classical and often effective simple reagent Ammonium sulfate in a Grid Screen format across the pH range 3.5 to 8.5. Classical salts Sodium chloride, Phosphate and Formate are also sampled across a broad range of pH. Successful crystallization screening in this zone of Index might indicate a Grid Screen optimization using a simple salt versus pH approach might be useful for optimization and production of crystals.

Neutralized organic acids have recently been reported as highly effective crystallization reagents. These Index salts include Malonate, Citrate, Succinate, Malate, Formate, Acetate, Tartrate, and Tacsimate™.

Relatively high supersaturation levels of salts combined with low concentration of polymers are reported as effective crystallization reagent systems and are found in the Index reagents 30-36.

Some proteins, especially intact and fragmented antibodies respond well to low ionic strength crystallization reagent systems which are found in Index reagents 37-48.

Non-volatile organics such as MPD as well as polyols such as PEG 400 and low molecular weight PEG MME are frequently reported in the literature as useful reagents in the crystallization of nucleic acids and these reagents are also effective with proteins, especially when combined with salts. More recently, a class of reagents, pentaerythritols, has been reported as effective crystallization reagents.

Currently, if one were to select the most reported precipitant system successful in producing single crystals of biological macromolecules, it would be the combination of high purity Polyethylene glycols with salts. Between 1999 and 2002, 60% of the crystallization reported in the literature utilized a Polyethylene glycol / salt reagent formulation. More than 30 Index conditions evaluate this highly effective reagent combination across a broad pH range.

Index is formulated in zones. Classic salts versus pH. Neutralized organic acids. High [salt] with low [polymer]. High [polymer] with low [salt]. Low ionic strength versus pH. PEG & Salt versus pH. PEG & Salt. If one zone is more effective at producing crystals than another zone, then further crystallization screening and/or optimization could or should focus on this

reagent system zone. Zone formulation makes interpretation of screen results a bit easier and much faster.

Many of the Index reagent formulations were selected from the literature based on the reagent's relative efficacy in producing crystals of biological macromolecular crystals. Other reagents were selected and in a single case, synthesized based on their unique chemical properties, their compatibility with Micro Batch, Vapor and Liquid Diffusion methods, and their ability to produce crystals where at times, the other reagent systems failed. These reagent systems, not strongly represented in the literature were evaluated at Hampton Research using a portfolio of more than 40 biological macromolecules. Finally, a sampling of the Index formulations were designed using the Incomplete Factorial approach and again evaluated at Hampton Research using a portfolio of more than 40 biological macromolecules. Further evaluation of Index was performed in collaboration with academic and industrial crystallography labs in order to test and refine the Index formulation. Each reagent formulation in Index has produced a crystal of a biological macromolecule.

Sample Preparation

The macromolecular sample should be homogenous, as pure as is practically possible (>95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or microfiltration prior to use.

The recommended sample concentration is 5 to 25 mg/ml in sterile filtered, dilute (25 mM or less) buffer. For initial screens, the sample should be free of unnecessary additives in order to observe the effect of the Index and Index 2 variables. However, agents that promote and preserve sample stability and homogeneity can and should be included in the sample. For additional sample preparation recommendation see Crystal Growth 101 - Preliminary Sample Preparation bulletin from Hampton Research.

Preparing the Deep Well Block for Use

It is recommended the Deep Well block be centrifuged before removing the sealing film. Centrifugation at 500 rpm for five minutes will remove stray reagent from the sealing film. Removing the reagent from the film prevents stray reagent droplets from falling into neighboring wells during film removal. After centrifugation the film can be removed by grasping a corner of the film and gently peeling the film from the plate. Alternatively, the film can be left intact and the pierced for reagent access.

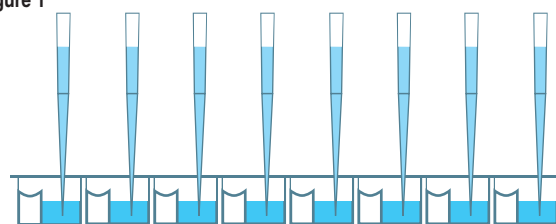
Performing the Screen

Manual Method - Sitting Drop Vapor Diffusion

1. Using a 96 well sitting drop vapor diffusion plate, pipet the recommended volume (typically 50 to 100 microliters) of crystallization reagent from the Deep Well block into the reservoirs of the crystallization plate. The Deep Well block is compatible with 8 and 12 channel pipets as well as many au-

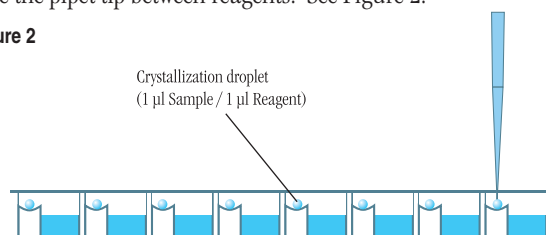
tomated liquid handling systems. Use clean pipet tips for each reagent set transfer and change pipet tips when changing reagents. For an 8 channel pipet, transfer reagents A1-H1 to reservoirs A1-H1 of the crystallization plate. Repeat this procedure for reagent columns B through H. Change pipet tips when moving between reagent columns. For a 12 channel pipet, transfer reagents A1-A12 to reservoirs A1-A12 of the crystallization plate. Repeat this procedure for reagent rows 1 through 12. See Figure 1. Time and pipet tips can be conserved by batch pipetting multiple plates with the same (row or column) of reagent before changing reagent and pipet tips.

Figure 1



2. Using clean pipet tips, pipet 0.05 to 2 microliters of crystallization reagent from the crystallization plate reservoir to the sitting drop well. Some 96 well crystallization plates allow this procedure to be performed using a multi-channel pipet where other plates require the use of a single channel pipet. Change the pipet tip between reagents. See Figure 2.

Figure 2



3. Using a clean pipet tip, pipet 0.05 to 2 microliters of sample to the reagent drop in the sitting drop well. One may choose to simply dispense the sample with no mixing or dispense with mixing by gently aspirating and dispensing the sample several times, keeping the tip in the drop during mixing to avoid foaming. Work carefully but quickly to minimize evaporation from the crystallization plate. See Figure 2 above.

4. Seal the crystallization plate as per the manufacturer's recommendation. Most 96 well crystallization plates are sealed using a clear sealing tape or film. View and score the experiment as desired. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

5. Seal the remaining reagent in the Deep Well block using either clear sealing tape, film, or cap mat.

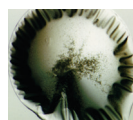
Manual Method – Micro Batch 96 well format

1. Using a 96 well clear polystyrene microplate (U-bottom recommended

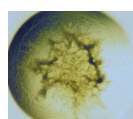
Figure 6
Typical observations in a crystallization experiment



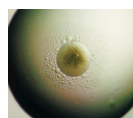
Clear Drop



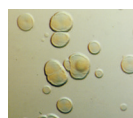
Skin /
Precipitate



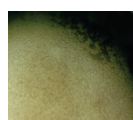
Precipitate



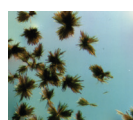
Precipitate /
Phase



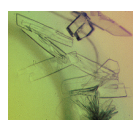
Quasi
Crystals



Microcrystals



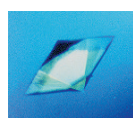
Needle
Cluster



Plates



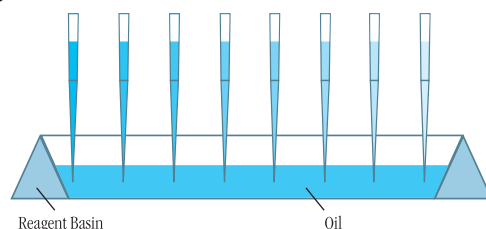
Rod Cluster



Single
Crystal

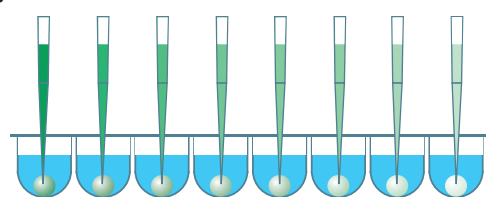
for best drop centering, flat-bottom recommended for best optics) pipet 50 to 150 microliters of microbatch compatible oil into each of the 96 reservoirs. This can be accomplished using an 8 or 12 channel pipet and pipetting the oil from a reagent basin. See Figure 3.

Figure 3



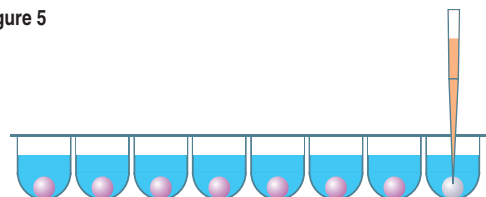
2. Once the plate is oiled, use an 8 or 12 channel pipet to aspirate reagent from the Deep Well block and dispense the reagent under the oil in the MicroBatch plate. Change tips when changing reagent to prevent cross reagent contamination. To save time and pipet tips, set multiple plates at one time. See Figure 4.

Figure 4



3. Using a single channel pipet, aspirate the sample and dispense the sample under oil in the MicroBatch plate. It is not necessary to dispense the sample drop into the reagent drop or mix the drops. See Figure 5.

Figure 5



4. After all reagent and sample drops have been dispensed to the MicroBatch plate, place the loose fitting clear cover on the MicroBatch plate and centrifuge the plate for 10 minutes at 500 rpm. Centrifugation will cause the drops to coalesce into a single drop.

Note: If the drops appear flat or is fragmented into multiple drops, the centrifugation speed is too high and the centrifugation time is too long - adjust to obtain a spherical single drop in the center of the well.

5. Store the plates with the loose fitting clear polystyrene cover and observe for crystals. See Hampton Research technical bulletin Crystal Growth 101 - Viewing Crystallization Experiments for additional information on viewing drops.

Index HT Deep Well Block and Automated Liquid Handling Systems

The polypropylene Deep Well block is designed to be compatible with the SBS standard 96 microwell format and is therefore compatible with numerous automated liquid handling systems that accept 8x12 96 well assay blocks. Follow the manufacturer's recommendation for handling deep well microplates.

Examine the Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week thereafter. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 6, on the left side of page 3 shows typical examples of what one might observe in a crystallization experiment.

Interpreting Index HT

Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If the drop remains clear after 3 to 4 weeks consider repeating the screen condition and doubling the sample concentration. If more than 70 of the 96 screen drops are clear consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the screen condition. If more than 70 of the 96 screen drops contain precipitate and no crystals are present, consider diluting the sample concentration in half and repeating the entire screen. If sample denaturation is suspect, take measures to

stabilize the sample (add reducing agent, ligands, glycerol, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing optics to differentiate precipitate from microcrystalline material.

If the drop contains a macromolecular crystal the relative supersaturation of the sample and reagent is appropriate for crystal nucleation and growth. The next step is to optimize the preliminary conditions (pH, salt type, salt concentration, precipitant type, precipitant concentration, sample concentration, temperature, additives, and other crystallization variables) which produced the crystal in order to improve crystal size and quality.

Compare the observations between the 4°C and room temperature incubation to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

Retain and observe plates until the drops are dried out. Crystal growth can occur within 15 minutes or one year.

Index HT Formulation

Crystallization reagents are formulated using the highest purity chemicals, ultrapure water (18.2 Megohm-cm, 5 ppb TOC) and are sterile filtered using 0.22 micron filters into sterile Deep Well blocks (no preservatives added).

Crystallization reagents are readily reproduced using Hampton Research Optimize™ and StockOptions™ stock solutions of salts, polymers and buffers. Optimize and StockOptions stock reagents make reproducing crystallization screen reagents accurate, precise, fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize and StockOptions stock reagents.

Crystallization reagents containing buffers are formulated by creating a 1.0 M stock buffer, titrated to the desired pH using Hydrochloric acid or Sodium hydroxide. The buffer is then diluted with the other reagent components and water. No further pH adjustment is required.

Crystallization reagents are stable at room temperature and are best if used within 12 months of receipt. To enhance reagent stability it is strongly recommended that crystallization reagents be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

If the sample contains phosphate, borate, or carbonate buffers it is possible to obtain inorganic crystals (false positives) when using crystallization reagents containing divalent cations such as magnesium, calcium, or zinc.

To avoid false positives use phosphate, borate, or carbonate buffers at concentrations of 10 mM or less or exchange the phosphate, borate, or carbonate buffer with a more soluble buffer that does not complex with divalent cations.

References and Readings

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Technical Support

Inquiries regarding Index HT reagent formulation, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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How to Reproduce Index HT Reagents

Index HT reagents and optimization conditions based on Index HT hits can be formulated using volumetric methods and carefully prepared reagent stocks (Table 1). Note the examples below.

Example 1. To prepare 1.0 milliliter of Index HT reagent 4 (A4) in a crystallization plate.

Solution Composition: 0.1 M BIS-TRIS pH 6.5
2.0 M Ammonium sulfate

- 329 µl water³
- 100 µl 1.0 M BIS-TRIS pH 6.5
(CAS # 6976-37-0, Catalog # HR2-783)
- 571 µl 3.5 M Ammonium sulfate
(CAS # 7783-20-2, Catalog # HR2-541)

Make no pH adjustments. Mix well by aspirating and dispensing the solution multiple times.

Example 2. To prepare 1.0 milliliter of Index HT reagent 17 (B5).

Solution Composition: 1.26 M Sodium phosphate monobasic monohydrate,
0.14 M Potassium phosphate dibasic, pH 5.6

- 650 µl water³
- 35 µl 4.0 M Potassium phosphate dibasic
(CAS # 7758-11-4, Catalog # HR2-635)
- 315 µl 4.0 M Sodium phosphate monobasic monohydrate
(CAS # 10049-21-5, Catalog # HR2-551)

Make no pH adjustments. Mix well. Final pH will be 5.6

Example 3. To prepare 10 milliliters of Index HT reagent 25 (C1).

Solution Composition: 3.5 M Sodium formate pH 7.0

- 3.0 ml water³
- 7.0 ml 5.0 M Sodium formate pH 7.0
(CAS # 141-53-7, Catalog # HR2-765)

Make no pH adjustments. Mix well.

³ ASTM Type II (laboratory grade) or Type III (analytical grade) water.

Formulation Notes for Index HT Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce an Index HT reagent.
2. All Optimize solutions and screen reagents are sterile filtered using 0.22 µm filters into sterile containers.

3. Add water first as this will help maintain the solubility of subsequently added reagents.
4. When formulating reagents using a pipet, add the largest volume last (except water). Use this larger volume setting to aspirate and dispense the reagent until the solution is mixed.
5. When formulating reagents using a pipet, use a clean, sterile pipet tip for each reagent added to the solution.
6. Use the buffers in Table 2 to systematically vary the pH as a crystallization variable.

pH as a Crystallization Variable

The buffers listed in Table 2, can be used to vary the pH as a crystallization variable and are recommended when optimizing a crystal grown from an Index HT kit.

Optimize™ buffer stocks are supplied as a 100 milliliters sterile filtered solution. Optimize buffers are available as an acid-base pair or titrated to a specific pH.

StockOptions™ buffer kits contain 10 milliliters each of ready to pipet buffers, titrated in 0.1 pH increments over the indicated pH range. The number of reagents offered in a StockOptions buffer kit depends upon the pH range of the buffer. The broader the pH range, the more buffers in the kit.

Online Information

Visit www.hamptonresearch.com and enter one of the following:

- Reagent Catalog Number
- Kit Catalog Number
- CAS Number
- Reagent Name

To obtain reagent specifications, pH titration tables, user guides, certificates of analysis, material safety data sheets (MSDS), and any other additional information.

MakeTray™

MakeTray is a free, web based program at www.hamptonresearch.com which generates both a pipetting worksheet and a reagent formulation document for crystallization set ups. MakeTray allows one to enter general information about the sample and experiment, which is then printed on the pipet worksheet and the reagent formulation document. The plate size can be customized for any number of wells, so MakeTray works for: 24, 48, and 96 well plates. MakeTray is especially useful for the design and formulation of crystal optimization experiments.

Table 1. Recommended reagents for the formulation of Index HT and Optimization reagents.

Each of these reagents are available as an Optimize™ crystallization grade reagent from Hampton Research. Table 1 provides the common chemical name, the Hampton Research catalog number, supplied stock concentration, the supplied volume, and the CAS number for each reagent. For more information on a specific Optimize reagent, go to

www.hamptonresearch.com. Using Search, enter either the catalog number, CAS number, or chemical name to obtain additional information for the Optimize reagent, including a Certificate of Analysis and MSDS (where applicable).

| Salts | Hampton Research Catalog # | Supplied [Stock] | Supplied Volume | CAS # |
|--|----------------------------|--------------------|-----------------|-------------|
| Ammonium acetate | HR2-565 | 1.0 M | 100 ml | 631-61-8 |
| | HR2-799 | 8.0 M | 200 ml | 631-61-8 |
| Ammonium citrate tribasic pH 7.0 | HR2-759 | 2.5 M | 200 ml | 3458-72-8 |
| Ammonium sulfate | HR2-541 | 3.5 M | 200 ml | 7783-20-2 |
| Ammonium tartrate dibasic pH 7.0 | HR2-767 | 1.6 M | 200 ml | 3164-29-2 |
| Cadmium chloride hydrate | HR2-715 | 1.0 M | 100 ml | 654054-66-7 |
| Calcium chloride dihydrate | HR2-557 | 2.0 M | 100 ml | 10035-04-8 |
| Cobalt(II) chloride hexahydrate | HR2-713 | 1.0 M | 100 ml | 7791-13-1 |
| Lithium sulfate monohydrate | HR2-545 | 2.0 M | 200 ml | 10102-25-7 |
| Magnesium chloride hexahydrate | HR2-559 | 2.0 M | 100 ml | 7791-18-6 |
| | HR2-803 | 5.0 M | 200 ml | 7791-18-6 |
| Magnesium formate dihydrate | HR2-537 | 1.0 M | 200 ml | 557-39-1 |
| DL-Malic acid pH 7.0 | HR2-761 | 3.0 M | 200 ml | 6915-15-7 |
| Nickel(II) chloride hexahydrate | HR2-687 | 4.0 M | 200 ml | 7791-20-0 |
| Potassium bromide | HR2-779 | 4.0 M | 100 ml | 7758-02-3 |
| Potassium chloride | HR2-649 | 4.0 M | 200 ml | 7447-40-7 |
| Potassium phosphate dibasic | HR2-635 | 4.0 M | 200 ml | 7758-11-4 |
| Potassium sodium tartrate tetrahydrate | HR2-539 | 1.5 M | 200 ml | 6381-59-5 |
| Potassium thiocyanate | HR2-695 | 8.0 M | 200 ml | 333-20-0 |
| L-Proline | HR2-775 | 1.0 M | 100 ml | 147-85-3 |
| Sodium acetate trihydrate pH 7.0 | HR2-763 | 4.0 M | 200 ml | 6131-90-4 |
| Sodium chloride | HR2-637 | 5.0 M | 200 ml | 7647-14-5 |
| Sodium citrate tribasic dihydrate | HR2-549 | 1.6 M | 200 ml | 6132-04-3 |
| Sodium formate | HR2-547 | 7.0 M | 200 ml | 141-53-7 |
| Sodium formate pH 7.0 | HR2-765 | 5.0 M | 200 ml | 141-53-7 |
| Sodium malonate pH 7.0 | HR2-707 | 3.4 M | 200 ml | 141-82-2 |
| Sodium phosphate monobasic monohydrate | HR2-551 | 4.0 M | 200 ml | 10049-21-5 |
| Succinic acid pH 7.0 | HR2-709 | 1.2 M | 200 ml | 110-15-6 |
| Tacsimate pH 7.0 | HR2-755 | 100% | 200 ml | N/A |
| Trimethylamine N-oxide dihydrate | HR2-777 | 1.0 M | 100 ml | 62637-93-8 |
| Zinc acetate dihydrate | HR2-563 | 1.0 M | 100 ml | 5970-45-6 |

Table 1 (Continued). Recommended reagents for the formulation of Index HT and Optimization reagents.

| Polymers | Hampton Research Catalog # | Supplied [Stock] | Supplied Volume | CAS # |
|--|----------------------------|--------------------|-----------------|------------|
| Jeffamine® M-600® pH 7.0 | HR2-501 | 50% v/v | 200 ml | 77110-54-4 |
| Jeffamine® ED-2001 pH 7.0 | HR2-597 | 50% w/v | 200 ml | 65605-36-9 |
| Pentaerythritol propoxylate (5/4 PO/OH) | HR2-739 | 50% v/v | 200 ml | 9051-49-4 |
| Pentaerythritol ethoxylate (15/4 EO/OH) | HR2-745 | 50% v/v | 200 ml | 30599-15-6 |
| Polyethylene glycol P 400 | HR2-771 | 100% | 200 ml | 25322-69-4 |
| Poly(acrylic acid sodium salt) 5,100 | HR2-773 | 50% w/v | 200 ml | 9003-04-7 |
| Polyethylene glycol 1,500 | HR2-525 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 3,350 | HR2-527 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 8,000 | HR2-535 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol 10,000 | HR2-607 | 50% w/v | 200 ml | 25322-68-3 |
| Polyethylene glycol monomethyl ether 550 | HR2-611 | 100% | 200 ml | 9004-74-4 |
| Polyethylene glycol monomethyl ether 2,000 | HR2-613 | 50% w/v | 200 ml | 9004-74-4 |
| Polyethylene glycol monomethyl ether 5,000 | HR2-615 | 50% w/v | 200 ml | 9004-74-4 |
| Polyvinylpyrrolidone K 15 | HR2-769 | 5% w/v | 200 ml | 9003-39-8 |
| | | | | |
| Organics (non-volatile) | Hampton Research Catalog # | Supplied [Stock] | Supplied Volume | CAS # |
| (+/-)-2-Methyl-2,4-pentanediol | HR2-627 | 100% | 200 ml | 107-41-5 |
| | | | | |
| Buffers | Hampton Research Catalog # | Supplied [Stock] | Supplied Volume | CAS # |
| BIS-TRIS pH 5.5 ¹ | HR2-781 | 1.0 M | 100 ml | 6976-37-0 |
| BIS-TRIS pH 6.5 ¹ | HR2-783 | 1.0 M | 100 ml | 6976-37-0 |
| Citric acid pH 3.5 ² | HR2-757 | 1.0 M | 100 ml | 77-92-9 |
| HEPES pH 7.0 ² | HR2-785 | 1.0 M | 100 ml | 7365-45-9 |
| HEPES pH 7.5 ² | HR2-729 | 1.0 M | 100 ml | 7365-45-9 |
| Sodium acetate trihydrate pH 4.5 ¹ | HR2-789 | 1.0 M | 100 ml | 6131-90-4 |
| Tris pH 8.5 ¹ | HR2-725 | 1.0 M | 100 ml | 77-86-1 |
| | | | | |
| ¹ pH titrated using Hydrochloric acid (HR2-581) CAS # 7647-01-0 | | | | |
| ² pH titrated using Sodium hydroxide (HR2-583) CAS # 1310-73-2 | | | | |

Table 2. Recommended buffers for screening the pH of Index HT and Optimization reagents.

| Buffer Solution <u>or</u> Kit | Hampton Research Catalog # | Supplied [Stock] | Supplied Volume | CAS # | pH range |
|---|----------------------------|--------------------|-----------------|-----------|-----------|
| StockOptions™ Bis-Tris kit ⁴ | HR2-106 | 1.0 M | 10 ml each | 6976-37-0 | 5.5 - 7.5 |
| StockOptions™ Citric Acid kit ⁴ | HR2-104 | 1.0 M | 10 ml each | 77-92-9 | 2.2 - 6.5 |
| HEPES <u>untitrated</u> | HR2-585 | 1.0 M | 100 ml | 7365-45-9 | 6.6 - 8.5 |
| Titrate with NaOH | HR2-583 | 1.0 M | 100 ml | 1310-73-2 | — |
| StockOptions™ Hepes kit ⁴ | HR2-102 | 1.0 M | 10 ml each | 7365-45-9 | 6.8 - 8.2 |
| Sodium acetate trihydrate <u>untitrated</u> | HR2-569 | 1.0 M | 100 ml | 6131-90-4 | 3.6 - 5.6 |
| Titrate with HCl | HR2-581 | 1.0 M | 100 ml | 7647-01-0 | — |
| StockOptions™ Sodium Acetate kit ⁴ | HR2-233 | 1.0 M | 10 ml each | 6131-90-4 | 3.6 - 5.6 |
| Tris <u>untitrated</u> | HR2-589 | 1.0 M | 100 ml | 77-86-1 | 7.0 - 9.0 |
| Titrate with HCl | HR2-581 | 1.0 M | 100 ml | 7647-01-0 | — |
| StockOptions™ Tris kit ⁴ | HR2-100 | 1.0 M | 10 ml each | 77-86-1 | 7.0 - 9.0 |
| ⁴ Individual StockOptions buffers titrated to any pH within the kit's pH range are available in 185 ml volumes from the Hampton Research Custom Shop | | | | | |

Technical Support

Inquiries regarding Index HT Fundamentals, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

Jeffamine® is a registered trademark of the Huntsman Petrochemical Corporation.

M-600® is a registered trademark of Texaco.

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| Well # | Salt | Well # | Buffer ◊ | Well # | Precipitant |
|-----------|--|-----------|--|-----------|--|
| 1. (A1) | None | 1. (A1) | 0.1 M Citric acid pH 3.5 | 1. (A1) | 2.0 M Ammonium sulfate |
| 2. (A2) | None | 2. (A2) | 0.1 M Sodium acetate trihydrate pH 4.5 | 2. (A2) | 2.0 M Ammonium sulfate |
| 3. (A3) | None | 3. (A3) | 0.1 M BIS-TRIS pH 5.5 | 3. (A3) | 2.0 M Ammonium sulfate |
| 4. (A4) | None | 4. (A4) | 0.1 M BIS-TRIS pH 6.5 | 4. (A4) | 2.0 M Ammonium sulfate |
| 5. (A5) | None | 5. (A5) | 0.1 M HEPES pH 7.5 | 5. (A5) | 2.0 M Ammonium sulfate |
| 6. (A6) | None | 6. (A6) | 0.1 M Tris pH 8.5 | 6. (A6) | 2.0 M Ammonium sulfate |
| 7. (A7) | None | 7. (A7) | 0.1 M Citric acid pH 3.5 | 7. (A7) | 3.0 M Sodium chloride |
| 8. (A8) | None | 8. (A8) | 0.1 M Sodium acetate trihydrate pH 4.5 | 8. (A8) | 3.0 M Sodium chloride |
| 9. (A9) | None | 9. (A9) | 0.1 M BIS-TRIS pH 5.5 | 9. (A9) | 3.0 M Sodium chloride |
| 10. (A10) | None | 10. (A10) | 0.1 M BIS-TRIS pH 6.5 | 10. (A10) | 3.0 M Sodium chloride |
| 11. (A11) | None | 11. (A11) | 0.1 M HEPES pH 7.5 | 11. (A11) | 3.0 M Sodium chloride |
| 12. (A12) | None | 12. (A12) | 0.1 M Tris pH 8.5 | 12. (A12) | 3.0 M Sodium chloride |
| 13. (B1) | None | 13. (B1) | 0.1 M BIS-TRIS pH 5.5 | 13. (B1) | 0.3 M Magnesium formate dihydrate |
| 14. (B2) | None | 14. (B2) | 0.1 M BIS-TRIS pH 6.5 | 14. (B2) | 0.5 M Magnesium formate dihydrate |
| 15. (B3) | None | 15. (B3) | 0.1 M HEPES pH 7.5 | 15. (B3) | 0.5 M Magnesium formate dihydrate |
| 16. (B4) | None | 16. (B4) | 0.1 M Tris pH 8.5 | 16. (B4) | 0.3 M Magnesium formate dihydrate |
| 17. (B5) | None | 17. (B5) | None - pH 5.6 | 17. (B5) | 1.26 M Sodium phosphate monobasic monohydrate, 0.14 M Potassium phosphate dibasic |
| 18. (B6) | None | 18. (B6) | None - pH 6.9 | 18. (B6) | 0.49 M Sodium phosphate monobasic monohydrate, 0.91 M Potassium phosphate dibasic |
| 19. (B7) | None | 19. (B7) | None - pH 8.2 | 19. (B7) | 0.056 M Sodium phosphate monobasic monohydrate, 1.344 M Potassium phosphate dibasic |
| 20. (B8) | None | 20. (B8) | 0.1 M HEPES pH 7.5 | 20. (B8) | 1.4 M Sodium citrate tribasic dihydrate |
| 21. (B9) | None | 21. (B9) | None | 21. (B9) | 1.8 M Ammonium citrate tribasic pH 7.0 |
| 22. (B10) | None | 22. (B10) | None | 22. (B10) | 0.8 M Succinic acid pH 7.0 |
| 23. (B11) | None | 23. (B11) | None | 23. (B11) | 2.1 M DL-Malic acid pH 7.0 |
| 24. (B12) | None | 24. (B12) | None | 24. (B12) | 2.8 M Sodium acetate trihydrate pH 7.0 |
| 25. (C1) | None | 25. (C1) | None | 25. (C1) | 3.5 M Sodium formate pH 7.0 |
| 26. (C2) | None | 26. (C2) | None | 26. (C2) | 1.1 M Ammonium tartrate dibasic pH 7.0 |
| 27. (C3) | None | 27. (C3) | None | 27. (C3) | 2.4 M Sodium malonate pH 7.0 |
| 28. (C4) | None | 28. (C4) | None | 28. (C4) | 35% v/v Tacsimate pH 7.0 |
| 29. (C5) | None | 29. (C5) | None | 29. (C5) | 60% v/v Tacsimate pH 7.0 |
| 30. (C6) | 0.1 M Sodium chloride | 30. (C6) | 0.1 M BIS-TRIS pH 6.5 | 30. (C6) | 1.5 M Ammonium sulfate |
| 31. (C7) | 0.8 M Potassium sodium tartrate tetrahydrate | 31. (C7) | 0.1 M Tris pH 8.5 | 31. (C7) | 0.5% w/v Polyethylene glycol monomethyl ether 5,000 |
| 32. (C8) | 1.0 M Ammonium sulfate | 32. (C8) | 0.1 M BIS-TRIS pH 5.5 | 32. (C8) | 1% w/v Polyethylene glycol 3,350 |
| 33. (C9) | 1.1 M Sodium malonate pH 7.0 | 33. (C9) | 0.1 M HEPES pH 7.0 | 33. (C9) | 0.5% v/v Jeffamine® ED-2001 pH 7.0 |
| 34. (C10) | 1.0 M Succinic acid pH 7.0 | 34. (C10) | 0.1 M HEPES pH 7.0 | 34. (C10) | 1% w/v Polyethylene glycol monomethyl ether 2,000 |
| 35. (C11) | 1.0 M Ammonium sulfate | 35. (C11) | 0.1 M HEPES pH 7.0 | 35. (C11) | 0.5% w/v Polyethylene glycol 8,000 |
| 36. (C12) | 15% v/v Tacsimate pH 7.0 | 36. (C12) | 0.1 M HEPES pH 7.0 | 36. (C12) | 2% w/v Polyethylene glycol 3,350 |
| 37. (D1) | None | 37. (D1) | None | 37. (D1) | 25% w/v Polyethylene glycol 1,500 |
| 38. (D2) | None | 38. (D2) | 0.1 M HEPES pH 7.0 | 38. (D2) | 30% v/v Jeffamine® M-600® pH 7.0 |
| 39. (D3) | None | 39. (D3) | 0.1 M HEPES pH 7.0 | 39. (D3) | 30% v/v Jeffamine® ED-2001 pH 7.0 |
| 40. (D4) | None | 40. (D4) | 0.1 M Citric acid pH 3.5 | 40. (D4) | 25% w/v Polyethylene glycol 3,350 |
| 41. (D5) | None | 41. (D5) | 0.1 M Sodium acetate trihydrate pH 4.5 | 41. (D5) | 25% w/v Polyethylene glycol 3,350 |
| 42. (D6) | None | 42. (D6) | 0.1 M BIS-TRIS pH 5.5 | 42. (D6) | 25% w/v Polyethylene glycol 3,350 |
| 43. (D7) | None | 43. (D7) | 0.1 M BIS-TRIS pH 6.5 | 43. (D7) | 25% w/v Polyethylene glycol 3,350 |
| 44. (D8) | None | 44. (D8) | 0.1 M HEPES pH 7.5 | 44. (D8) | 25% w/v Polyethylene glycol 3,350 |
| 45. (D9) | None | 45. (D9) | 0.1 M Tris pH 8.5 | 45. (D9) | 25% w/v Polyethylene glycol 3,350 |
| 46. (D10) | None | 46. (D10) | 0.1 M BIS-TRIS pH 6.5 | 46. (D10) | 20% w/v Polyethylene glycol monomethyl ether 5,000 |
| 47. (D11) | None | 47. (D11) | 0.1 M BIS-TRIS pH 6.5 | 47. (D11) | 28% w/v Polyethylene glycol monomethyl ether 2,000 |
| 48. (D12) | 0.2 M Calcium chloride dihydrate | 48. (D12) | 0.1 M BIS-TRIS pH 5.5 | 48. (D12) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |

◊ Buffer pH is that of a 1.0 M stock prior to dilution with other reagent components:
pH with HCl or NaOH.

Index HT contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

| Well # | Salt | Well # | Buffer ◇ | Well # | Precipitant |
|-----------|---|-----------|------------------------|-----------|--|
| 49. (E1) | 0.2 M Calcium chloride dihydrate | 49. (E1) | 0.1 M BIS-TRIS pH 6.5 | 49. (E1) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 50. (E2) | 0.2 M Ammonium acetate | 50. (E2) | 0.1 M BIS-TRIS pH 5.5 | 50. (E2) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 51. (E3) | 0.2 M Ammonium acetate | 51. (E3) | 0.1 M BIS-TRIS pH 6.5 | 51. (E3) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 52. (E4) | 0.2 M Ammonium acetate | 52. (E4) | 0.1 M HEPES pH 7.5 | 52. (E4) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 53. (E5) | 0.2 M Ammonium acetate | 53. (E5) | 0.1 M Tris pH 8.5 | 53. (E5) | 45% v/v (+/-)-2-Methyl-2,4-pentanediol |
| 54. (E6) | 0.05 M Calcium chloride dihydrate | 54. (E6) | 0.1 M BIS-TRIS pH 6.5 | 54. (E6) | 30% v/v Polyethylene glycol monomethyl ether 550 |
| 55. (E7) | 0.05 M Magnesium chloride hexahydrate | 55. (E7) | 0.1 M HEPES pH 7.5 | 55. (E7) | 30% v/v Polyethylene glycol monomethyl ether 550 |
| 56. (E8) | 0.2 M Potassium chloride | 56. (E8) | 0.05 M HEPES pH 7.5 | 56. (E8) | 35% v/v Pentaerythritol propoxylate (5/4 PO/OH) |
| 57. (E9) | 0.05 M Ammonium sulfate | 57. (E9) | 0.05 M BIS-TRIS pH 6.5 | 57. (E9) | 30% v/v Pentaerythritol ethoxylate (15/4 EO/OH) |
| 58. (E10) | None | 58. (E10) | 0.1 M BIS-TRIS pH 6.5 | 58. (E10) | 45% v/v Polypropylene glycol P 400 |
| 59. (E11) | 0.02 M Magnesium chloride hexahydrate | 59. (E11) | 0.1 M HEPES pH 7.5 | 59. (E11) | 22% w/v Poly(acrylic acid sodium salt) 5,100 |
| 60. (E12) | 0.01 M Cobalt(II) chloride hexahydrate | 60. (E12) | 0.1 M Tris pH 8.5 | 60. (E12) | 20% w/v Polyvinylpyrrolidone K 15 |
| 61. (F1) | 0.2 M L-Proline | 61. (F1) | 0.1 M HEPES pH 7.5 | 61. (F1) | 10% w/v Polyethylene glycol 3,350 |
| 62. (F2) | 0.2 M Trimethylamine N-oxide dihydrate | 62. (F2) | 0.1 M Tris pH 8.5 | 62. (F2) | 20% w/v Polyethylene glycol monomethyl ether 2,000 |
| 63. (F3) | 5% v/v Tacsimate pH 7.0 | 63. (F3) | 0.1 M HEPES pH 7.0 | 63. (F3) | 10% w/v Polyethylene glycol monomethyl ether 5,000 |
| 64. (F4) | 0.005 M Cobalt(II) chloride hexahydrate, 0.005 M Nickel(II) chloride hexahydrate, 0.005 M Cadmium chloride hydrate, 0.005 M Magnesium chloride hexahydrate | 64. (F4) | 0.1 M HEPES pH 7.5 | 64. (F4) | 12% w/v Polyethylene glycol 3,350 |
| 65. (F5) | 0.1 M Ammonium acetate | 65. (F5) | 0.1 M BIS-TRIS pH 5.5 | 65. (F5) | 17% w/v Polyethylene glycol 10,000 |
| 66. (F6) | 0.2 M Ammonium sulfate | 66. (F6) | 0.1 M BIS-TRIS pH 5.5 | 66. (F6) | 25% w/v Polyethylene glycol 3,350 |
| 67. (F7) | 0.2 M Ammonium sulfate | 67. (F7) | 0.1 M BIS-TRIS pH 6.5 | 67. (F7) | 25% w/v Polyethylene glycol 3,350 |
| 68. (F8) | 0.2 M Ammonium sulfate | 68. (F8) | 0.1 M HEPES pH 7.5 | 68. (F8) | 25% w/v Polyethylene glycol 3,350 |
| 69. (F9) | 0.2 M Ammonium sulfate | 69. (F9) | 0.1 M Tris pH 8.5 | 69. (F9) | 25% w/v Polyethylene glycol 3,350 |
| 70. (F10) | 0.2 M Sodium chloride | 70. (F10) | 0.1 M BIS-TRIS pH 5.5 | 70. (F10) | 25% w/v Polyethylene glycol 3,350 |
| 71. (F11) | 0.2 M Sodium chloride | 71. (F11) | 0.1 M BIS-TRIS pH 6.5 | 71. (F11) | 25% w/v Polyethylene glycol 3,350 |
| 72. (F12) | 0.2 M Sodium chloride | 72. (F12) | 0.1 M HEPES pH 7.5 | 72. (F12) | 25% w/v Polyethylene glycol 3,350 |
| 73. (G1) | 0.2 M Sodium chloride | 73. (G1) | 0.1 M Tris pH 8.5 | 73. (G1) | 25% w/v Polyethylene glycol 3,350 |
| 74. (G2) | 0.2 M Lithium sulfate monohydrate | 74. (G2) | 0.1 M BIS-TRIS pH 5.5 | 74. (G2) | 25% w/v Polyethylene glycol 3,350 |
| 75. (G3) | 0.2 M Lithium sulfate monohydrate | 75. (G3) | 0.1 M BIS-TRIS pH 6.5 | 75. (G3) | 25% w/v Polyethylene glycol 3,350 |
| 76. (G4) | 0.2 M Lithium sulfate monohydrate | 76. (G4) | 0.1 M HEPES pH 7.5 | 76. (G4) | 25% w/v Polyethylene glycol 3,350 |
| 77. (G5) | 0.2 M Lithium sulfate monohydrate | 77. (G5) | 0.1 M Tris pH 8.5 | 77. (G5) | 25% w/v Polyethylene glycol 3,350 |
| 78. (G6) | 0.2 M Ammonium acetate | 78. (G6) | 0.1 M BIS-TRIS pH 5.5 | 78. (G6) | 25% w/v Polyethylene glycol 3,350 |
| 79. (G7) | 0.2 M Ammonium acetate | 79. (G7) | 0.1 M BIS-TRIS pH 6.5 | 79. (G7) | 25% w/v Polyethylene glycol 3,350 |
| 80. (G8) | 0.2 M Ammonium acetate | 80. (G8) | 0.1 M HEPES pH 7.5 | 80. (G8) | 25% w/v Polyethylene glycol 3,350 |
| 81. (G9) | 0.2 M Ammonium acetate | 81. (G9) | 0.1 M Tris pH 8.5 | 81. (G9) | 25% w/v Polyethylene glycol 3,350 |
| 82. (G10) | 0.2 M Magnesium chloride hexahydrate | 82. (G10) | 0.1 M BIS-TRIS pH 5.5 | 82. (G10) | 25% w/v Polyethylene glycol 3,350 |
| 83. (G11) | 0.2 M Magnesium chloride hexahydrate | 83. (G11) | 0.1 M BIS-TRIS pH 6.5 | 83. (G11) | 25% w/v Polyethylene glycol 3,350 |
| 84. (G12) | 0.2 M Magnesium chloride hexahydrate | 84. (G12) | 0.1 M HEPES pH 7.5 | 84. (G12) | 25% w/v Polyethylene glycol 3,350 |
| 85. (H1) | 0.2 M Magnesium chloride hexahydrate | 85. (H1) | 0.1 M Tris pH 8.5 | 85. (H1) | 25% w/v Polyethylene glycol 3,350 |
| 86. (H2) | 0.2 M Potassium sodium tartrate tetrahydrate | 86. (H2) | None | 86. (H2) | 20% w/v Polyethylene glycol 3,350 |
| 87. (H3) | 0.2 M Sodium malonate pH 7.0 | 87. (H3) | None | 87. (H3) | 20% w/v Polyethylene glycol 3,350 |
| 88. (H4) | 0.2 M Ammonium citrate tribasic pH 7.0 | 88. (H4) | None | 88. (H4) | 20% w/v Polyethylene glycol 3,350 |
| 89. (H5) | 0.1 M Succinic acid pH 7.0 | 89. (H5) | None | 89. (H5) | 15% w/v Polyethylene glycol 3,350 |
| 90. (H6) | 0.2 M Sodium formate | 90. (H6) | None | 90. (H6) | 20% w/v Polyethylene glycol 3,350 |
| 91. (H7) | 0.15 M DL-Malic acid pH 7.0 | 91. (H7) | None | 91. (H7) | 20% w/v Polyethylene glycol 3,350 |
| 92. (H8) | 0.1 M Magnesium formate dihydrate | 92. (H8) | None | 92. (H8) | 15% w/v Polyethylene glycol 3,350 |
| 93. (H9) | 0.05 M Zinc acetate dihydrate | 93. (H9) | None | 93. (H9) | 20% w/v Polyethylene glycol 3,350 |
| 94. (H10) | 0.2 M Sodium citrate tribasic dihydrate | 94. (H10) | None | 94. (H10) | 20% w/v Polyethylene glycol 3,350 |
| 95. (H11) | 0.1 M Potassium thiocyanate | 95. (H11) | None | 95. (H11) | 30% w/v Polyethylene glycol monomethyl ether 2,000 |
| 96. (H12) | 0.15 M Potassium bromide | 96. (H12) | None | 96. (H12) | 30% w/v Polyethylene glycol monomethyl ether 2,000 |

◇ Buffer pH is that of a 1.0 M stock prior to dilution
with other reagent components:
pH with HCl or NaOH.

Index HT contains ninety-six unique reagents. To determine the formulation of each reagent, simply read across the page.

Sample: _____ Sample Concentration: _____
 Sample Buffer: _____ Date: _____
 Reservoir Volume: _____ Temperature: _____
 Drop Volume: Total _____ µl Sample _____ µl Reservoir _____ µl Additive _____ µl

- 1 Clear Drop
- 2 Phase Separation
- 3 Regular Granular Precipitate
- 4 Birefringent Precipitate or Microcrystals

- 5 Posettes or Spherulites
- 6 Needles (1D Growth)
- 7 Plates (2D Growth)
- 8 Single Crystals (3D Growth < 0.2 mm)
- 9 Single Crystals (3D Growth > 0.2 mm)

Index HT™ - HR2-134 Scoring Sheet

Date: Date: Date:

| | | | | |
|-----------|---|--|--|--|
| 49. (E1) | 0.2 M Calcium chloride dihydrate, 0.1 M BIS-TRIS pH 6.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 50. (E2) | 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 51. (E3) | 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 6.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 52. (E4) | 0.2 M Ammonium acetate, 0.1 M HEPES pH 7.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 53. (E5) | 0.2 M Ammonium acetate, 0.1 M Tris pH 8.5, 45% v/v (+/-)-2-Methyl-2,4-pentanediol | | | |
| 54. (E6) | 0.05 M Calcium chloride dihydrate, 0.1 M BIS-TRIS pH 6.5, 30% v/v Polyethylene glycol monomethyl ether 550 | | | |
| 55. (E7) | 0.05 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 30% v/v Polyethylene glycol monomethyl ether 550 | | | |
| 56. (E8) | 0.2 M Potassium chloride, 0.05 M HEPES pH 7.5, 35% v/v Pentaerythritol propoxylate (5/4 PO/OH) | | | |
| 57. (E9) | 0.05 M Ammonium sulfate, 0.05 M BIS-TRIS pH 6.5, 30% v/v Pentaerythritol ethoxylate (15/4 EO/OH) | | | |
| 58. (E10) | 0.1 M BIS-TRIS pH 6.5, 45% v/v Polypropylene glycol P 400 | | | |
| 59. (E11) | 0.02 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 22% w/v Poly(acrylic acid sodium salt) 5,100 | | | |
| 60. (E12) | 0.01 M Cobalt(II) chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyvinylpyrrolidone K 15 | | | |
| 61. (F1) | 0.2 M L-Proline, 0.1 M HEPES pH 7.5, 10% w/v Polyethylene glycol 3,350 | | | |
| 62. (F2) | 0.2 M Trimethylamine N-oxide dihydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 63. (F3) | 5% v/v Tacsimate pH 7.0, 0.1 M HEPES pH 7.0, 10% w/v Polyethylene glycol monomethyl ether 5,000 | | | |
| 64. (F4) | 0.005 M Cobalt(II) chloride hexahydrate, 0.005 M Nickel(II) chloride hexahydrate, 0.005 M Cadmium chloride hydrate, 0.005 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 12% w/v Polyethylene glycol 3,350 | | | |
| 65. (F5) | 0.1 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 17% w/v Polyethylene glycol 10,000 | | | |
| 66. (F6) | 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 67. (F7) | 0.2 M Ammonium sulfate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 68. (F8) | 0.2 M Ammonium sulfate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 69. (F9) | 0.2 M Ammonium sulfate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 70. (F10) | 0.2 M Sodium chloride, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 71. (F11) | 0.2 M Sodium chloride, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 72. (F12) | 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 73. (G1) | 0.2 M Sodium chloride, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 74. (G2) | 0.2 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 75. (G3) | 0.2 M Lithium sulfate monohydrate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 76. (G4) | 0.2 M Lithium sulfate monohydrate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 77. (G5) | 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 78. (G6) | 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 79. (G7) | 0.2 M Ammonium acetate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 80. (G8) | 0.2 M Ammonium acetate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 81. (G9) | 0.2 M Ammonium acetate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 82. (G10) | 0.2 M Magnesium chloride hexahydrate, 0.1 M BIS-TRIS pH 5.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 83. (G11) | 0.2 M Magnesium chloride hexahydrate, 0.1 M BIS-TRIS pH 6.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 84. (G12) | 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 85. (H1) | 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 25% w/v Polyethylene glycol 3,350 | | | |
| 86. (H2) | 0.2 M Potassium sodium tartrate tetrahydrate, 20% w/v Polyethylene glycol 3,350 | | | |
| 87. (H3) | 0.2 M Sodium malonate pH 7.0, 20% w/v Polyethylene glycol 3,350 | | | |
| 88. (H4) | 0.2 M Ammonium citrate tribasic pH 7.0, 20% w/v Polyethylene glycol 3,350 | | | |
| 89. (H5) | 0.1 M Succinic acid pH 7.0, 15% w/v Polyethylene glycol 3,350 | | | |
| 90. (H6) | 0.2 M Sodium formate, 20% w/v Polyethylene glycol 3,350 | | | |
| 91. (H7) | 0.15 M DL-Malic acid pH 7.0, 20% w/v Polyethylene glycol 3,350 | | | |
| 92. (H8) | 0.1 M Magnesium formate dihydrate, 15% w/v Polyethylene glycol 3,350 | | | |
| 93. (H9) | 0.05 M Zinc acetate dihydrate, 20% w/v Polyethylene glycol 3,350 | | | |
| 94. (H10) | 0.2 M Sodium citrate tribasic dihydrate, 20% w/v Polyethylene glycol 3,350 | | | |
| 95. (H11) | 0.1 M Potassium thiocyanate, 30% w/v Polyethylene glycol monomethyl ether 2,000 | | | |
| 96. (H12) | 0.15 M Potassium bromide, 30% w/v Polyethylene glycol monomethyl ether 2,000 | | | |