Wizard Screen 2

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-105 (pg 1)**

Wizard Screen 2 is a complete reagent kit designed to provide a rapid screening method for the crystallization of biological macromolecules using a large range of precipitants, buffers, and salts, covering a broad range of crystallization space at pH levels from pH 4.5 to pH 10.5. Wizard Screen 2 is a straightforward, effective, and practical kit for determining preliminary crystallization conditions. Wizard Screen 2 is also effective in determining the solubility of a macromolecule in a wide range of precipitants and pH.

Wizard Screen 2 is a sparse matrix of trial crystallization reagent conditions developed by Steve L. Sarfaty and Wim G. J. Hol. The primary screen variables are salt, pH, and precipitant (salts, polymers, volatile organics, and non-volatile organics).

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 micro-filtration prior to use (1, 2, 3).

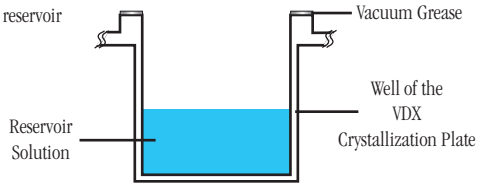
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Wizard Screen 2 variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against water although ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, or activity.

Performing The Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of Wizard Screen 2 with the Hanging Drop Vapor Diffusion method. Wizard Screen 2 is also very compatible with the Sitting Drop, Sandwich Drop, MicroBatch, and Microdialysis methods. A complete description of the Hanging, Sitting, Sandwich Drop, Dialysis and other crystallization methods are available from the Hampton Research Crystal Growth 101 Library.

1. Prepare a VDX Plate (HR3-141) for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. One may also use a Greased VDX Plate (HR3-171). Forty eight reservoirs are to be prepared for a complete Wizard Screen 2. See Figure 1.

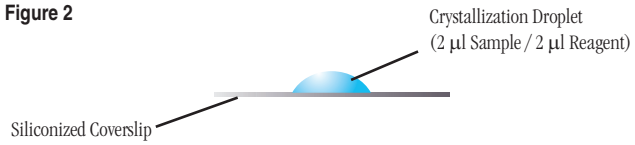
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Wizard Screen 2 reagent 1 into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of Wizard Screen 2 reagent 2 into reservoir A2. Repeat the procedure for the remaining 46 Wizard Screen 2 reagents using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

3. Pipet 2 µl of the sample to the center of a clean, siliconized 22 mm diameter circle or square cover slide. See Figure 2.

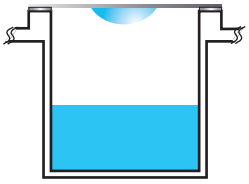
Figure 2



4. Pipet 2 µl of Wizard Screen 2 reagent 1 from reservoir A1 into the sample droplet and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during mixing to avoid foaming. See Figure 2.

5. Working quickly to minimize evaporation, invert the cover slide and droplet over reservoir A1 and seal the cover slide onto the edge of the reservoir. See Figure 3.

Figure 3
Inverted siliconized coverslip placed over the reservoir.



6. Repeat operations 3 through 5 for the remaining 47 Wizard Screen 2 reagents.

7. If the quantity of sample permits, perform Wizard Screen 2 in duplicate and incubate one set of plates at 4°C and the second set at room temperature. Incubate and store the crystallization plates in a stable temperature environment free of vibration.

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 there after. 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 4 (on page 2) shows typical examples of what one might observe in a crystallization experiment.

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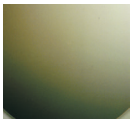
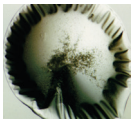
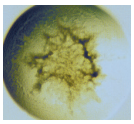
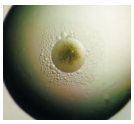
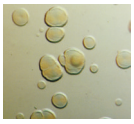
Figure 4
Typical observations in a crystallization experiment

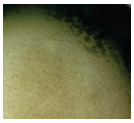

Clear Drop


Skin / Precipitate

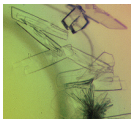

Precipitate


Precipitate / Phase

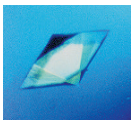

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Interpreting Wizard Screen 2

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 Wizard Screen 2 condition and doubling the sample concentration. If more than 35 of the 48 Wizard Screen 2 drops are clear consider doubling the sample concentration and repeating the entire screen.

Drops containing precipitate indicate that 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 Wizard Screen 2 condition. If more than 35 of the 48 Wizard Screen 2 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 good. 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.

Wizard Screen 2 Formulation

Wizard Screen 2 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 containers (no preservatives added).

Wizard Screen 2 reagents are readily reproduced using Hampton Research Optimize™ stock solutions of salts, polymers and buffers. Optimize stock reagents make reproducing Wizard Screen 2 reagents fast, convenient and easy. Dilutions can be performed directly into the crystallization plate using Optimize stock reagents.

Wizard Screen 2 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.

Wizard Screen 2 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 Wizard Screen 2 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 Wizard Screen 2 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

1. Crystallization of nucleic acids and proteins, Edited by A. Ducruix and R. Giege, The Practical Approach Series, Oxford Univ. Press, 1992.

2. Current approaches to macromolecular crystallization. McPherson, A. Eur. J. Biochem. 189, 1-23, 1990.

3. Protein and Nucleic Acid Crystallization. Methods, A Companion to Methods in Enzymology, Academic Press, Volume 1, Number 1, August 1990.

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Technical Support

Inquiries regarding Wizard Screen 2 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 4:00 p.m. USA Pacific Standard Time.

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Fundamentals **HR2-105**

How to Reproduce Wizard Screen 2 Reagents

Wizard Screen 2 reagents and optimization conditions based on Wizard Screen 2 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 Wizard Screen 2 reagent 8 in a crystallization plate.

Solution Composition: 10% w/v Polyethylene glycol 8,000,
0.1 M Sodium phosphate monobasic monohydrate / Potassium phosphate dibasic pH 6.2,
0.2 M Sodium chloride

- 735 µl water[3]
- 200 µl 50% w/v Polyethylene glycol 8,000 (CAS # 25322-68-3, Catalog # HR2-535)
- 40 µl 5.0 M Sodium chloride (CAS # 7647-14-5, Catalog # HR2-637)
- 17 µl 4.0 M Sodium phosphate monobasic monohydrate (CAS # 10049-21-5, Catalog# HR2-551)
- 8 µl 4.0 M Potassium phosphate dibasic (CAS # 7758-11-4, Catalog# HR2-635)

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

Example 2. To prepare 10 milliliters of Wizard Screen 2 reagent 31.

Solution Composition: 1.0 M Sodium citrate tribasic dihydrate,
0.1 M Tris pH 7.0,
0.2 M Sodium chloride

- 2.3 ml water[3]
- 6.3 ml 1.6 M Sodium citrate tribasic dihydrate (CAS # 6132-04-3, Catalog# HR2-549)
- 1.0 ml 1.0 M Tris pH 7.0 (CAS # 77-86-1, Catalog# HR2-900-01)
- 0.4 ml 5.0 M Sodium chloride (CAS # 7647-14-5, Catalog# HR2-637)

Make no pH adjustments. Mix well.

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

Formulation Notes for Wizard Screen 2 Reagents

1. No additional pH adjustment is made to any reagent after formulation. Use the buffers in Table 1 to reproduce an Wizard Screen 2 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 Wizard Screen 2 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 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, safety data sheets (SDS), and any other additional information.

MakeTray™

MakeTray is a free, web based program at 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.

Technical Support

Inquiries regarding Wizard Screen 2 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 4:00 p.m. USA Pacific Standard Time.

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Fundamentals HR2-105

Table 1. Recommended reagents for the formulation of Wizard Screen 2 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 SDS (where applicable).

Salts	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
Ammonium phosphate dibasic	HR2-629	3.5 M	200 ml	7783-28-0
Ammonium sulfate	HR2-541	3.5 M	200 ml	7783-20-2
Calcium acetate hydrate	HR2-567	1.0 M	100 ml	114460-21-8
Lithium sulfate monohydrate	HR2-545	2.0 M	200 ml	10377-48-7
Magnesium chloride hexahydrate	HR2-559	2.0 M	100 ml	7791-18-6
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
Sodium acetate trihydrate	HR2-543	3.0 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 phosphate monobasic monohydrate	HR2-551	4.0 M	200 ml	10049-21-5
Zinc acetate dihydrate	HR2-563	1.0 M	100 ml	5970-45-6
Polymers	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
Polyethylene glycol 400	HR2-603	100 %	200 ml	25322-68-3
Polyethylene glycol 1,000	HR2-523	50 % w/v	200 ml	25322-68-3
Polyethylene glycol 3,000	HR2-604	50% w/v	200 ml	25322-68-3
Polyethylene glycol 8,000	HR2-535	50% w/v	200 ml	25322-68-3
Organics (non-volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
(+/-)-2-Methyl-2,4-pentanediol	HR2-627	100%	200 ml	107-41-5
1,4-Butanediol	N/A	N/A	N/A	110-63-4
Organics (volatile)	**Hampton Research Catalog #**	**Supplied [Stock]**	**Supplied Volume**	**CAS #**
2-Propanol	N/A	N/A	N/A	67-63-0
Ethanol	N/A	N/A	N/A	64-17-5

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Table 1 (Continued). **Recommended reagents for the formulation of Wizard Screen 2 and Optimization reagents.**

Buffers	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #
CAPS pH 10.5 [2]	HR2-941-42-5	1.0 M	185 ml	1135-40-6
CHES pH 9.5 [1]	HR2-256-10	1.0 M	185 ml	103-47-9
HEPES pH 7.5 [2]	HR2-729	1.0 M	100 ml	7365-45-9
Imidazole pH 8.0 [1]	HR2-995-19	1.0 M	185 ml	288-32-4
MES monohydrate pH 6.0 [2]	HR2-943-09	1.0 M	185 ml	145224-94-8
Sodium acetate trihydrate pH 4.5 [1]	HR2-789	1.0 M	100 ml	6131-90-4
Sodium cacodylate trihydrate pH 6.5 [1]	HR2-737	1.0 M	100 ml	6131-99-3
Sodium citrate tribasic dihydrate pH 4.2 [1]	HR2-935-01	1.0 M	185 ml	6132-04-3
Sodium citrate tribasic dihydrate pH 5.5 [1]	HR2-935-14	1.0 M	185 ml	6132-04-3
Tris pH 7.0 [1]	HR2-900-01	1.0 M	185 ml	77-86-1
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 Wizard Screen 2 and Optimization reagents.**

Buffer Solution or Kit	Hampton Research Catalog #	Supplied [Stock]	Supplied Volume	CAS #	pH range
StockOptions™ CHES kit [4]	HR2-256	1.0 M	10 ml each	103-47-9	8.6 - 10.0
StockOptions™ Imidazole kit [4]	HR2-095	1.0 M	10 ml each	288-32-4	6.2 - 7.8
StockOptions™ Hepes kit [4]	HR2-102	1.0 M	10 ml each	7365-45-9	6.8 - 8.2
StockOptions™ MES kit [4]	HR2-243	1.0 M	10 ml each	145224-94-8	5.2 - 7.1
StockOptions™ Sodium Acetate kit [4]	HR2-233	1.0 M	10 ml each	6131-90-4	3.6 - 5.6
StockOptions™ Sodium Cacodylate kit [4]	HR2-239	1.0 M	10 ml each	6131-99-3	5.1 - 7.4
StockOptions™ Sodium Citrate kit [4]	HR2-235	1.0 M	10 ml each	6132-04-3	4.2 - 6.5
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					

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HR2-105 Reagent Formulation

Tube #	Salt	Buffer ◊	Precipitant
1.	0.2 M Zinc acetate dihydrate	0.1 M Sodium acetate trihydrate pH 4.5	10% w/v Polyethylene glycol 3,000
2.	0.2 M Lithium sulfate monohydrate	0.1 M MES monohydrate pH 6.0	35% v/v (+/-)-2-Methyl-2,4-pentanediol
3.	0.2 M Magnesium chloride hexahydrate	0.1 M Tris pH 8.5	20% w/v Polyethylene glycol 8,000
4.	0.2 M Sodium chloride	0.1 M Sodium cacodylate trihydrate pH 6.5	2.0 M Ammonium sulfate
5.	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	20% v/v 1,4-Butanediol
6.	0.2 M Lithium sulfate monohydrate	0.1 M Sodium citrate tribasic dihydrate pH 4.2	10% v/v 2-Propanol
7.	0.2 M Sodium chloride	0.1 M Tris pH 7.0	30% w/v Polyethylene glycol 3,000
8.	0.2 M Sodium chloride	00.068 M Sodium phosphate monobasic monohydrate, 0.032 M Potassium phosphate dibasic pH 6.2	10% w/v Polyethylene glycol 8,000
9.	None	0.1 M Sodium citrate tribasic dihydrate pH 4.2	2.0 M Ammonium sulfate
10.	None	0.1 M Tris pH 8.5	1.0 M Ammonium phosphate dibasic
11.	0.2 M Zinc acetate dihydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	10% v/v 2-Propanol
12.	0.2 M Lithium sulfate monohydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	30% v/v Polyethylene glycol 400
13.	0.2 M Lithium sulfate monohydrate	0.1 M Sodium citrate tribasic dihydrate pH 5.5	15% v/v Ethanol
14.	0.2 M Sodium chloride	00.068 M Sodium phosphate monobasic monohydrate, 0.032 M Potassium phosphate dibasic pH 6.2	20% w/v Polyethylene glycol 1,000
15.	None	0.1 M HEPES pH 7.5	1.26 M Ammonium sulfate
16.	None	0.1 M CHES pH 9.5	1.0 M Sodium citrate tribasic dihydrate
17.	0.2 M Magnesium chloride hexahydrate	0.1 M Tris pH 7.0	2.5 M Sodium chloride
18.	0.2 M Calcium acetate hydrate	0.1 M Tris pH 7.0	20% w/v Polyethylene glycol 3,000
19.	None	0.1 M Sodium citrate tribasic dihydrate pH 4.2	0.4 M Potassium phosphate dibasic, 1.6 M Sodium phosphate monobasic monohydrate
20.	0.2 M Zinc acetate dihydrate	0.1 M MES monohydrate pH 6.0	15% v/v Ethanol
21.	None	0.1 M Sodium acetate trihydrate pH 4.5	35% v/v (+/-)-2-Methyl-2,4-pentanediol
22.	None	0.1 M Imidazole pH 8.0	10% v/v 2-Propanol
23.	0.2 M Magnesium chloride hexahydrate	0.1 M HEPES pH 7.5	15% v/v Ethanol
24.	0.2 M Sodium chloride	0.1 M Imidazole pH 8.0	30% w/v Polyethylene glycol 8,000
25.	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	35% v/v (+/-)-2-Methyl-2,4-pentanediol
26.	None	0.1 M CHES pH 9.5	30% v/v Polyethylene glycol 400
27.	0.2 M Magnesium chloride hexahydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	0% w/v Polyethylene glycol 3,000
28.	0.2 M Calcium acetate hydrate	0.1 M MES monohydrate pH 6.0	20% w/v Polyethylene glycol 8,000
29.	0.2 M Sodium chloride	0.1 M CHES pH 9.5	1.26 M Ammonium sulfate
30.	0.2 M Zinc acetate dihydrate	0.1 M Imidazole pH 8.0	20% v/v 1,4-Butanediol
31.	0.2 M Sodium chloride	0.1 M Tris pH 7.0	1.0 M Sodium citrate tribasic dihydrate
32.	None	0.1 M Tris pH 8.5	20% w/v Polyethylene glycol 1,000
33.	0.2 M Sodium chloride	0.1 M Sodium citrate tribasic dihydrate pH 5.5	1.0 M Ammonium phosphate dibasic
34.	None	0.1 M Imidazole pH 8.0	10% w/v Polyethylene glycol 8,000
35.	None	0.1 M Sodium acetate trihydrate pH 4.5	0.8 M Sodium phosphate monobasic monohydrate, 1.2 M Potassium phosphate dibasic
36.	0.2 M Sodium chloride	0.1 M Sodium citrate tribasic dihydrate pH 4.2	10% w/v Polyethylene glycol 3,000
37.	0.2 M Lithium sulfate monohydrate	0.1 M Tris pH 7.0	1.0 M Potassium sodium tartrate tetrahydrate
38.	0.2 M Lithium sulfate monohydrate	0.1 M Sodium acetate trihydrate pH 4.5	2.5 M Sodium chloride
39.	0.2 M Sodium chloride	0.1 M CAPS pH 10.5	20% w/v Polyethylene glycol 8,000
40.	0.2 M Zinc acetate dihydrate	0.1 M Imidazole pH 8.0	20% w/v Polyethylene glycol 3,000
41.	0.2 M Lithium sulfate monohydrate	0.1 M Tris pH 7.0	2.0 M Ammonium sulfate
42.	0.2 M Sodium chloride	0.1 M HEPES pH 7.5	30% v/v Polyethylene glycol 400
43.	0.2 M Magnesium chloride hexahydrate	0.1 M Tris pH 7.0	10% w/v Polyethylene glycol 8,000
44.	0.2 M Magnesium chloride hexahydrate	0.1 M Sodium cacodylate trihydrate pH 6.5	20% w/v Polyethylene glycol 1,000
45.	None	0.1 M MES monohydrate pH 6.	1.26 M Ammonium sulfate
46.	0.2 M Sodium chloride	0.1 M Imidazole pH 8.0	1.0 M Ammonium phosphate dibasic
47.	0.2 M Zinc acetate dihydrate	0.1 M Imidazole pH 8.0	2.5 M Sodium chloride
48.	None	0.1 M MES monohydrate pH 6.0	1.0 M Potassium sodium tartrate tetrahydrate

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

34 Journey
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HAMPTON RESEARCH
Solutions for Crystal Growth

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)

Wizard Screen 2 - HR2-105 Scoring Sheet

	Date:	Date:	Date:
1. (A1) 0.2 M Zinc acetate dihydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 10% w/v Polyethylene glycol 3,000			
2. (A2) 0.2 M Lithium sulfate monohydrate, 0.1 M MES monohydrate pH 6.0, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
3. (A3) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol 8,000			
4. (A4) 0.2 M Sodium chloride, 0.1 M Sodium cacodylate trihydrate pH 6.5, 2.0 M Ammonium sulfate			
5. (A5) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 20% v/v 1,4-Butanediol			
6. (A6) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 10% v/v 2-Propanol			
7. (A7) 0.2 M Sodium chloride, 0.1 M Tris pH 7.0, 30% w/v Polyethylene glycol 3,000			
8. (A8) 0.2 M NaCl, 0.1 M Na phosphate monobasic monohyd. / Potassium phosphate dibasic pH 6.2, 10% w/v PEG 8,000			
9. (A9) 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 2.0 M Ammonium sulfate			
10. (A10) 0.1 M Tris pH 8.5, 1.0 M Ammonium phosphate dibasic			
11. (A11) 0.2 M Zinc acetate dihydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 10% v/v 2-Propanol			
12. (A12) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 30% v/v Polyethylene glycol 400			
13. (B1) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 15% v/v Ethanol			
14. (B2) 0.2 M NaCl chloride, 0.1 M Na phosphate monobasic monohyd. / Potassium phosphate dibasic pH 6.2, 20% w/v PEG 1,000			
15. (B3) 0.1 M HEPES pH 7.5, 1.26 M Ammonium sulfate			
16. (B4) 0.1 M CHES pH 9.5, 1.0 M Sodium citrate tribasic dihydrate			
17. (B5) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 7.0, 2.5 M Sodium chloride			
18. (B6) 0.2 M Calcium acetate hydrate, 0.1 M Tris pH 7.0, 20% w/v Polyethylene glycol 3,000			
19. (B7) 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 0.4 M Potassium phosphate dibasic, 1.6 M Sodium phosphate monobasic monohydrate			
20. (B8) 0.2 M Zinc acetate dihydrate, 0.1 M MES monohydrate pH 6.0, 15% v/v Ethanol			
21. (B9) 0.1 M Sodium acetate trihydrate pH 4.5, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
22. (B10) 0.1 M Imidazole pH 8.0, 10% v/v 2-Propanol			
23. (B11) 0.2 M Magnesium chloride hexahydrate, 0.1 M HEPES pH 7.5, 15% v/v Ethanol			
24. (B12) 0.2 M Sodium chloride, 0.1 M Imidazole pH 8.0, 30% w/v Polyethylene glycol 8,000			
25. (C1) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 35% v/v (+/-)-2-Methyl-2,4-pentanediol			
26. (C2) 0.1 M CHES pH 9.5, 30% v/v Polyethylene glycol 400			
27. (C3) 0.2 M Magnesium chloride hexahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 10% w/v Polyethylene glycol 3,000			
28. (C4) 0.2 M Calcium acetate hydrate, 0.1 M MES monohydrate pH 6.0, 20% w/v Polyethylene glycol 8,000			
29. (C5) 0.2 M Sodium chloride, 0.1 M CHES pH 9.5, 1.26 M Ammonium sulfate			
30. (C6) 0.2 M Zinc acetate dihydrate, 0.1 M Imidazole pH 8.0, 20% v/v 1,4-Butanediol			
31. (C7) 0.2 M Sodium chloride, 0.1 M Tris pH 7.0, 1.0 M Sodium citrate tribasic dihydrate			
32. (C8) 0.1 M Tris pH 8.5, 20% w/v Polyethylene glycol 1,000			
33. (C9) 0.2 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 5.5, 1.0 M Ammonium phosphate dibasic			
34. (C10) 0.1 M Imidazole pH 8.0, 10% w/v Polyethylene glycol 8,000			
35. (C11) 0.1 M Sodium acetate trihydrate pH 4.5, 0.8 M Sodium phosphate monobasic monohyd., 1.2 M Potassium phosphate dibasic			
36. (C12) 0.2 M Sodium chloride, 0.1 M Sodium citrate tribasic dihydrate pH 4.2, 10% w/v Polyethylene glycol 3,000			
37. (D1) 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 7.0, 1.0 M Potassium sodium tartrate tetrahydrate			
38. (D2) 0.2 M Lithium sulfate monohydrate, 0.1 M Sodium acetate trihydrate pH 4.5, 2.5 M Sodium chloride			
39. (D3) 0.2 M Sodium chloride, 0.1 M CAPS pH 10.5, 20% w/v Polyethylene glycol 8,000			
40. (D4) 0.2 M Zinc acetate dihydrate, 0.1 M Imidazole pH 8.0, 20% w/v Polyethylene glycol 3,000			
41. (D5) 0.2 M Lithium sulfate monohydrate, 0.1 M Tris pH 7.0, 2.0 M Ammonium sulfate			
42. (D6) 0.2 M Sodium chloride, 0.1 M HEPES pH 7.5, 30% v/v Polyethylene glycol 400			
43. (D7) 0.2 M Magnesium chloride hexahydrate, 0.1 M Tris pH 7.0, 10% w/v Polyethylene glycol 8,000			
44. (D8) 0.2 M Magnesium chloride hexahydrate, 0.1 M Sodium cacodylate trihydrate pH 6.5, 20% w/v Polyethylene glycol 1,000			
45. (D9) 0.1 M MES monohydrate pH 6.0, 1.26 M Ammonium sulfate			
46. (D10) 0.2 M Sodium chloride, 0.1 M Imidazole pH 8.0, 1.0 M Ammonium phosphate dibasic			
47. (D11) 0.2 M Zinc acetate dihydrate, 0.1 M Imidazole pH 8.0, 2.5 M Sodium chloride			
48. (D12) 0.1 M MES monohydrate pH 6.0, 1.0 M Potassium sodium tartrate tetrahydrate			

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