Quik Optimize™

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-223 (pg 1)**

Quik Optimize™ is a reagent kit designed to provide a flexible phosphate reagent based screening method for the crystallization of biological macromolecules. The screen is simple and practical for finding initial crystallization conditions as well as determining the solubility of a macromolecule in Sodium and Potassium phosphate reagent system between pH 5.0 and 8.2. Quik Optimize is especially useful for refining and optimizing successful preliminary crystallization conditions determined using Quik Screen.

Using ultrapure water, Quik Optimize is composed of two high purity stock solutions.

- 4.0 M Sodium phosphate monobasic monohydrate, 100 ml ($NaH_2PO_4 \cdot H_2O$, M_r 137.99)
- 4.0 M Potassium phosphate dibasic, 100 ml (K_2HPO_4, M_r 174.18)

Final reagent pH is determined by the ratio of the mixture of the two phosphate salts in solution. The reagent concentration range that can be screened using Quik Optimize is 0.2 to 4.0 M (in 0.2 M increments) Na/K phosphate. The pH range of the screen is 5.0 to 8.2 (in 0.2 pH unit increments).

The Quik Optimize formulation has been used successfully in both small and large scale crystallizations for biological macromolecules. The phosphate system utilized by Quik Optimize is stable, safe, versatile, easy to reproduce, cost-effective and easy to scale up for large scale batch crystallization. Quik Optimize was developed by Macrocrystal Oy (Olarinluoma 16, Fin-02200 Espoo Finland) and is manufactured and distributed exclusively by Hampton Research.

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 Quik Optimize variables. Ideally, the initial screen should be performed with a sample which has been dialyzed against a dilute (25 mM) buffer, although ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, homogeneity, or activity.

Using Quik Optimize

Using the preformulated 4.0 M Sodium and Potassium phosphate stock solutions and the enclosed Quik Optimize Dilution Table one can conveniently formulate and screen Na / K phosphate concentrations between 0.2 and 4.0 M as well as pH values between 5 and 8.2.
The following steps below will help simplify the use of the Quik Optimize Dilution Table.

1. Find the desired reagent pH in the first or last columns in the top or bottom row of the Dilution Table.

2. Once the desired pH is determined, find the desired Na/K Phosphate concentration in the black outlined top or middle row of the Dilution Table.

3. Proceed down the Na/K phosphate concentration column and across the pH row until an intersection is formed. At the intersection, the amount of 4.0 M Na phosphate reagent stock, 4.0 M K phosphate reagent stock and water is defined which will formulate that reagent.

For example: To formulate 0.8 M Na/K phosphate pH 7.0 find the column 0.8 M Na/K and read down in the column until pH 7.0.

The Dilution Table instructs one to (Figure 1):

- Add 64 microliters of Na phosphate
- Add 136 microliters of K phosphate
- Add 800 microliters of water
- Thus creating 1.0 milliliter of 0.8 M Na/K phosphate pH 7.0 reagent.

Figure 1
Dilution Table example

pH	0.8 M Na/K		
5.0	196	4	800
5.2	195	5	800
5.4	189	11	800
5.6	180	20	800
5.8	168	32	800
6.0	153	47	800
6.2	137	63	800
6.4	119	81	800
6.6	100	100	800
6.8	82	118	800
7.0	**64**	**136**	**800**
7.2	48	152	800
7.4	34	166	800
7.6	22	178	800
7.8	13	187	800
8.0	9	191	800
8.2	8	192	800
	Na	K	H_2O

Performing the Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of Quik Screen with the Hanging Drop Vapor Diffusion method. Quik Screen 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.

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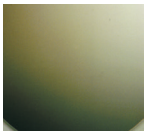
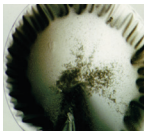
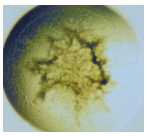
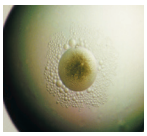
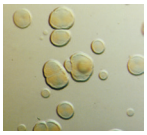
Figure 5
Typical observations in a crystallization experiment

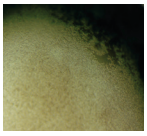

Clear Drop


Skin/Precipitate

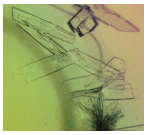

Precipitate


Precipitate/Phase

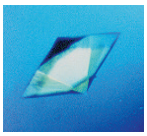

Quasi Crystals


Microcrystals


Needle Cluster

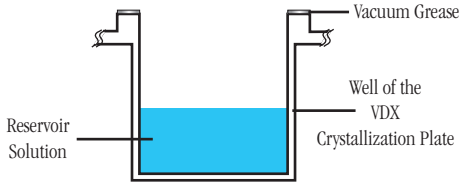

Plates


Rod Cluster


Single Crystal

1. Prepare a VDX Plate (HR3-140) 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 (HR3-170). See Figure 2.

Figure 2
Cross section of a reservoir in the VDX plate.

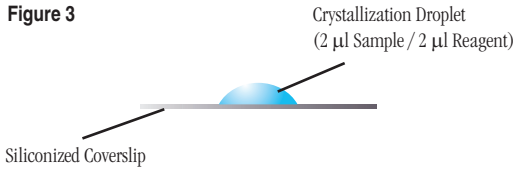


2. Using clean pipet tips, pipet of 1 ml of the desired Quik Optimize reagent combination into reservoir A1. Discard the pipet tip, add a new pipet tip and pipet 1 ml of the next desired Quik Optimize reagent combination into reservoir A2. Repeat the procedure for the remaining Quik Optimize reagent combinations using a clean pipet tip for each reagent so as to avoid reagent contamination and carry over.

(**Note**: One should be careful not to contaminate or dilute the Quik Optimize reagent stocks. Do this by using clean, dry pipet tips each time a new reagent is to be pipetted. One can conserve pipet tips by pipetting all of the Sodium phosphate additions, then changing pipet tips to perform the Potassium phosphate additions, then changing pipet tips to add water and mix the reagent. One may also choose to aliquot a small volume of the Quik Optimize reagent into a clean sterile container prior to pipetting the dilutions to further prevent contaminating the stock solutions).

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

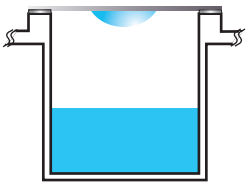
Figure 3



4. Pipet 2 µl (or less) of Quik Optimize reagent 1 from reservoir A1 into the sample droplet. See Figure 3.

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 4.

Figure 4
Inverted siliconized coverslip placed over the reservoir.



6. Repeat operations 3 through 5 for the remaining Quik Optimize reagent combinations.

7. If the quantity of sample permits, perform Quik Optimize 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 5 (on the left side of page 2), shows typical examples of what one might observe in a crystallization experiment.

Interpreting Quik Optimize

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 Quik Optimize condition and doubling the sample concentration. If more than 70% of the Quik Optimize drops are clear consider doubling the sample concentration and repeating the entire screen.

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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 Crystal Screen condition. If more than 70% of the Quik Optimize 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.

Quik Optimize Formulation

Quik Optimize 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).

Quik Optimize reagents are stable at room temperature.

If the sample contains divalent cations (magnesium, zinc, cadmium and calcium) it is possible to obtain inorganic crystals (false positives) when using Quik Optimize reagents. To avoid false positives use divalent cations in the sample at concentration of 10 mM or less or remove the cations by dialysis prior to screening with Quik Optimize.

Recommended Optimization Reagents

HR2-221 - Quik Screen Kit

The Quik Screen Kit features 24 unique reagents screening from 0.8 - 1.8 M Na/K phosphate versus pH 5.0 - 8.2 in a convenient grid format.

HR2-551 - 4.0 M Sodium phosphate monobasic monohydrate, 200 ml
M_r 137.99, $NaH_2PO_4 \cdot H_2O$, CAS [10049-21-5], EC No 231-449-2

HR2-635 - 4.0 M Potassium phosphate dibasic, 200 ml
M_r 174.18, K_2HPO_4, CAS [7758-11-4], EC No 231-834-5

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.

Technical Support

Inquiries regarding Quik Optimize Kit 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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