Quik Screen™

Sodium / Potassium Phosphate

HAMPTON RESEARCH
Solutions for Crystal Growth

User Guide **HR2-221**

Quik Screen™ is a preformulated reagent kit designed to provide a rapid 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 Screen is composed of high purity Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$, M_r 137.99), Potassium phosphate dibasic (K_2HPO_4, M_r 174.18), and ultrapure water. Reagent pH is determined by the ratio of the two salts in solution. The concentration range of the kit is 0.8 to 1.8 M (0.8, 1.0, 1.4, and 1.8 M) in phosphate. The pH range of the screen is 5.0 to 8.2 (5.0, 5.6, 6.3, 6.9, 7.5, and 8.2).

The Quik Screen formulation has been used successfully in both small and large scale crystallization of biological macromolecules. The phosphate system utilized by Quik Screen is stable, safe, versatile, easy to reproduce, cost-effective, and easy to scale up for large scale batch crystallization. Quik Screen 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). Since divalent cations can complex with the phosphate in Quik Screen and form inorganic salt crystals, the sample should be free of and contain less than 10mM of divalent cations such as: magnesium, calcium, and zinc.

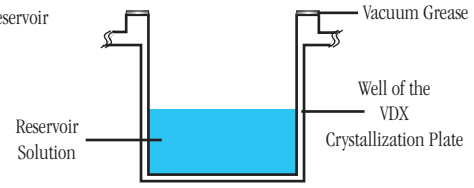
The recommended sample concentration is 5 to 25 mg/ml in water. Initially, the sample should be free of any unnecessary additives in order to observe the effect of the Quik Screen variables. Ideally, the initial screen should be preformed with a sample which has been dialyzed against water, although 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 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.

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). Twenty-four reservoirs are to be prepared for a complete Quik Screen. See Figure 1.

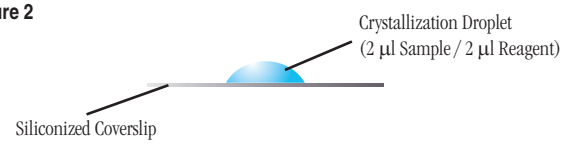
Figure 1
Cross section of a reservoir in the VDX plate.



2. Using a clean pipet tip, pipet 1 ml of Quik Screen reagent A1 into reservoir A1. Discard the pipet tip, add a new pipet tip, and pipet 1 ml of Quik Screen reagent A2 into reservoir A2. Repeat the procedure for the remaining 22 Quik Screen 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.

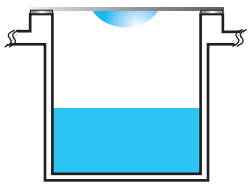
Figure 2



4. Pipet 2 µl of Quik Screen reagent A1 from reservoir A1 into the sample droplet and mix by aspirating and dispensing the droplet several times, keeping the tip in the drop during the 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 23 Quik Screen reagents.

7. If the quantity of sample permits, perform Quik Screen 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.

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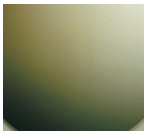
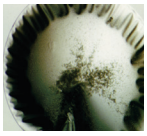
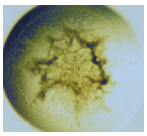
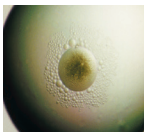
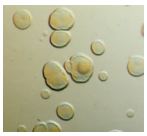
Figure 4
Typical observations in a crystallization experiment

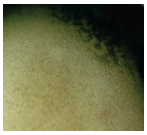

Clear Drop


Skin/Precipitate

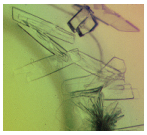

Precipitate


Precipitate/Phase

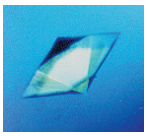

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

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 (left side of page 2) shows typical examples of what one might observe in a crystallization experiment.

Interpreting Quik Screen

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 Screen condition and doubling the sample concentration. If more than 70% Quik Screen 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 Quik Screen condition. If more than 70% Quik 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. 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 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 screen and optimization experiments.

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

Quik Screen Formulation

Quik Screen 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 Screen reagents are readily reproduced using Hampton Research Quik Optimize™ stock solutions. Quik Optimize stock reagents make reproducing Quik Screen reagents fast, convenient, and easy. Dilutions can be preformed directly into the crystallization plate using Quik Optimize stock reagents.

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

The pH values are approximate and may vary 0.2 pH units. The pH of the sodium potassium phosphate solution should not be adjusted with anything. The pH should be determined solely on the ratio of the two phosphate salts.

Quik Screen 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 Quik Screen be stored at 4°C or -20°C. Avoid ultraviolet light to preserve reagent stability.

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Recommended Optimization Reagents

HR2-223 - Quik Optimize kit

The Quik Optimize kit contains:

- 100 milliliters of 4.0 M Sodium phosphate monobasic monohydrate (M_r 137.99, $NaH_2PO_4 \cdot H_2O$, CAS [10049-21-5], EC No 231-449-2)
- 100 milliliters of 4.0 M Potassium phosphate dibasic (M_r 174.18, K_2HPO_4, CAS [7758-11-4], EC No 231-834-5)

An enclosed dilution table shows how to combine the two stock solution to create concentration ranges between 0.2 and 4.0 M and pH values between 5.0 and 8.2. This kit can be used to reproduce Quik Screen reagent conditions as well as formulate optimization conditions.

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 Screen 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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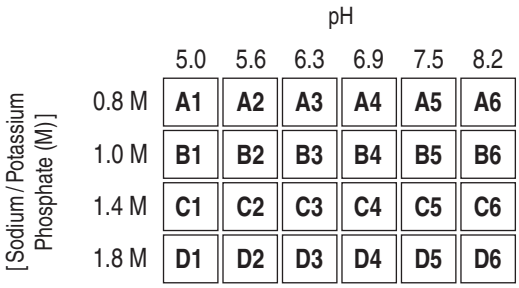

Tube Number	Sodium phosphate monobasic monohydrate [M]	Tube Number	Potassium phosphate dibasic[M]	Tube Number	Combined Concentration and pH
A1.	0.784	A1.	0.016	A1.	0.8 M Sodium / Potassium Phosphate pH 5.0
B1.	0.980	B1.	0.020	B1.	1.0 M Sodium / Potassium Phosphate pH 5.0
C1.	1.372	C1.	0.028	C1.	1.4 M Sodium / Potassium Phosphate pH 5.0
D1.	1.764	D1.	0.036	D1.	1.8 M Sodium / Potassium Phosphate pH 5.0
A2.	0.72	A2.	0.080	A2.	0.8 M Sodium / Potassium Phosphate pH 5.6
B2.	0.90	B2.	0.10	B2.	1.0 M Sodium / Potassium Phosphate pH 5.6
C2.	1.26	C2.	0.14	C2.	1.4 M Sodium / Potassium Phosphate pH 5.6
D2.	1.62	D2.	0.18	D2.	1.8 M Sodium / Potassium Phosphate pH 5.6
A3.	0.52	A3.	0.28	A3.	0.8 M Sodium / Potassium Phosphate pH 6.3
B3.	0.65	B3.	0.35	B3.	1.0 M Sodium / Potassium Phosphate pH 6.3
C3.	0.91	C3.	0.49	C3.	1.4 M Sodium / Potassium Phosphate pH 6.3
D3.	1.17	D3.	0.63	D3.	1.8 M Sodium / Potassium Phosphate pH 6.3
A4.	0.28	A4.	0.52	A4.	0.8 M Sodium / Potassium Phosphate pH 6.9
B4.	0.35	B4.	0.65	B4.	1.0 M Sodium / Potassium Phosphate pH 6.9
C4.	0.49	C4.	0.91	C4.	1.4 M Sodium / Potassium Phosphate pH 6.9
D4.	0.63	D4.	1.17	D4.	1.8 M Sodium / Potassium Phosphate pH 6.9
A5.	0.12	A5.	0.68	A5.	0.8 M Sodium / Potassium Phosphate pH 7.5
B5.	0.15	B5.	0.85	B5.	1.0 M Sodium / Potassium Phosphate pH 7.5
C5.	0.21	C5.	1.19	C5.	1.4 M Sodium / Potassium Phosphate pH 7.5
D5.	0.27	D5.	1.53	D5.	1.8 M Sodium / Potassium Phosphate pH 7.5
A6.	0.032	A6.	0.768	A6.	0.8 M Sodium / Potassium Phosphate pH 8.2
B6.	0.040	B6.	0.960	B6.	1.0 M Sodium / Potassium Phosphate pH 8.2
C6.	0.056	C6.	1.344	C6.	1.4 M Sodium / Potassium Phosphate pH 8.2
D6.	0.072	D6.	1.728	D6.	1.8 M Sodium / Potassium Phosphate pH 8.2

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An enclosed dilution table shows how to combine the two stock solution to create concentration ranges between 0.2 and 4.0 M and pH values between 5.0 and 8.2. This kit can be used to reproduce Quik Screen reagent conditions as well as formulate optimization conditions.

HR2-551 - 4.0 M Sodium phosphate monobasic monohydrate, 200 milliliters (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 milliliters (M_r 174.18, K_2HPO_4, CAS [7758-11-4], EC No 231-834-5)

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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)

Quik Screen™ - HR2-221 Scoring Sheet	Date:	Date:	Date:	Date:	Date:
A1. 0.8 M Sodium / Potassium Phosphate pH 5.0					
A2. 0.8 M Sodium / Potassium Phosphate pH 5.6					
A3. 0.8 M Sodium / Potassium Phosphate pH 6.3					
A4. 0.8 M Sodium / Potassium Phosphate pH 6.9					
A5. 0.8 M Sodium / Potassium Phosphate pH 7.5					
A6. 0.8 M Sodium / Potassium Phosphate pH 8.2					
B1. 1.0 M Sodium / Potassium Phosphate pH 5.0					
B2. 1.0 M Sodium / Potassium Phosphate pH 5.6					
B3. 1.0 M Sodium / Potassium Phosphate pH 6.3					
B4. 1.0 M Sodium / Potassium Phosphate pH 6.9					
B5. 1.0 M Sodium / Potassium Phosphate pH 7.5					
B6. 1.0 M Sodium / Potassium Phosphate pH 8.2					
C1. 1.4 M Sodium / Potassium Phosphate pH 5.0					
C2. 1.4 M Sodium / Potassium Phosphate pH 5.6					
C3. 1.4 M Sodium / Potassium Phosphate pH 6.3					
C4. 1.4 M Sodium / Potassium Phosphate pH 6.9					
C5. 1.4 M Sodium / Potassium Phosphate pH 7.5					
C6. 1.4 M Sodium / Potassium Phosphate pH 8.2					
D1. 1.8 M Sodium / Potassium Phosphate pH 5.0					
D2. 1.8 M Sodium / Potassium Phosphate pH 5.6					
D3. 1.8 M Sodium / Potassium Phosphate pH 6.3					
D4. 1.8 M Sodium / Potassium Phosphate pH 6.9					
D5. 1.8 M Sodium / Potassium Phosphate pH 7.5					
D6. 1.8 M Sodium / Potassium Phosphate pH 8.2					

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