Low Ionic Strength Screen™

HAMPTON RESEARCH
Solutions for Crystal Growth

Description

The Low Ionic Strength crystallization screen is based upon the screening protocol in the publication "Crystallization of intact monoclonal antibodies", Proteins: Structure, Function, and Genetics 23:285-289 (1995) by Lisa J. Harris, Eileen Skaletsky, and Alexander McPherson. The protocol is an effective screen for determining the preliminary crystallization conditions of intact monoclonal antibodies. However, this screen is not just an intact antibody screen. The screen has effectively determined the preliminary crystallization conditions for numerous monoclonal antibody fragments as well as other soluble proteins. The screen should be utilized as a low ionic strength crystallization screen for proteins where this strategy could be effective in determining preliminary crystallization screens.

In this screen, the concentration of a high purity, monodisperse PEG 3,350 is varied from 4 to 24% w/v (4, 8, 12, 16, 20 and 24% w/v) versus a pH range of 2 to 12 (2, 3, 3.5, 4, 4.5, 5, 5.5, 6.0, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12). Stock buffer concentrations are 50 mM. Final buffer concentration in the drop is typically 10 mM.

Unique Features of Low Ionic Strength Screen

1. Low ionic strength. Buffer concentration is supplied as 50 mM, resulting a an initial drop concentration of 10 mM (Drop = 4µl sample, 2 µl buffer, 5 µl precipitant).

2. The Polyethylene glycol (PEG) 3,350 is a special, high-purity, monodisperse preparation with a Mr of 3,300-3,400. Most PEGs of this molecular weight have an Mr of plus or minus 500 rather than 50.

3. An extremely broad range of pH 2 to 12 is sampled. At low ionic strength the effects of pH and temperature upon sample solubility are amplified. Hence this screen allows one to critically evaluate the effects of temperature and pH upon sample solubility and crystallization. It is recommended the screen be repeated at several temperatures between 4° and 37 °C to take advantage of the low ionic strength feature.

The protocol requires the following pipetting steps for a typical vapor diffusion experiment:

1. Pipet dehydrant to reservoir.

2. Pipet drop.

3. Pipet buffer to drop.

4. Pipet precipitant to drop.

Formulation

All solutions are formulated using ultra-pure chemicals and deionized water and are sterile filtered.

Storage

Recommended long term storage for the Low Ionic Strength Screen 24 unique reagents is -20°C. Allow the kit to equilibrate to room temperature prior to use.

Recommended storage for the 250 ml of dehydrant (24 % w/v PEG 3,350) supplied with the kit is -20° to 25°C.

Sample Preparation

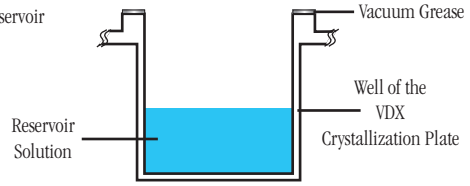
Samples should be highly purified and filtered using a 0.2 or 0.45 micron filter prior to crystallization screening. The sample should be suspended in deionized water or buffer of choice to a concentration of approximately 10 to 20 mg/ml. It is recommended that intact antibody solutions be dialyzed against deionized water and concentrated to 3 to 5 mg/ml. Centrifuge the sample to remove amorphous material prior to set up.

Performing the Screen

Since it is the most frequently reported method of crystallization, the following procedure describes the use of the Low Ionic Strength Screen with the Hanging Drop Vapor Diffusion method. Low Ionic Strength 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 for Hanging Drop Vapor Diffusion by applying a thin bead of cover slide sealant to the upper edge of each of the 24 reservoirs. See Figure 1.

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



2. Using a clean pipet tip, Pipet 1.0 milliliter of 24% w/v PEG 3,350 dehydrant into all of the reservoirs of the crystallization plate. If a slower equilibration rate or lower final equilibration concentration of PEG is desired, one may dilute the stock solution of 24% w/v PEG 3,350 to a lower concentration in the reservoir.

3. Pipet 4 µl of the sample to the center of a clean, siliconized 22 mm diameter cover slide. See Figure 2 below.

Figure 2

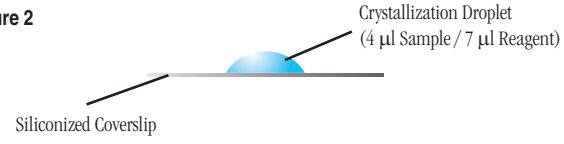


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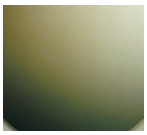
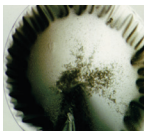
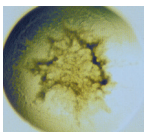
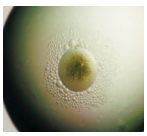
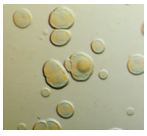
Figure 4
Typical observations in a crystallization experiment

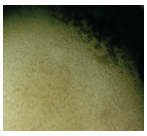

Clear Drop


Skin/Precipitate

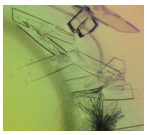

Precipitate


Precipitate/Phase

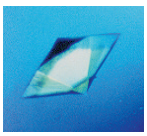

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster

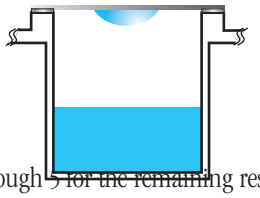

Single Crystal

4. Pipet 2 µl of buffer reagent (1-18) into the sample droplet, pipet 5 µl of PEG 3,350 precipitant (A - F) to the drop.

Note: Be sure to pipet Low Ionic Strength Screen reagents/precipitants into sample drops and **NOT** the dehydrant and mix by aspirating and dispensing the droplet several times, keeping the pipet tip in the drop during mixing to avoid foaming. See Figure 2 on page 1.

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 below.

Figure 3
Inverted siliconized coverslip placed over the reservoir.



6. Repeat operations 3 through 5 for the remaining reservoirs. It is possible to generate up to 108 unique precipitant/pH combinations if one were to screen pH 2 to 12 and PEG 3,350 concentrations of 4 to 24%.

7. If the quantity of sample permits, incubation temperature is 4, 18, 23 (room temperature), and 37°C. One need not screen all temperatures at once by performing four unique set ups unless sufficient sample is available for screening. One may perform the screen at one temperature and evaluate other temperatures based upon initial results. One significant advantage of the low ionic strength formulation is that temperature effects on sample solubility are exaggerated compared to higher ionic strength screens (Crystal Screen, Crystal Screen 2).

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 bi-pyramid 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 the left side of page 2) shows typical examples of what one might observe in a crystallization experiment.

Interpreting The Results

The sparse matrix screen used in the Low Ionic Strength Screen is designed to screen sample solubility as well as determine preliminary crystallization conditions.

When crystals are obtained during the initial screen, conditions may be optimized by varying the concentration of the precipitant (PEG 3,350) the molecular weight of the precipitant, the pH, temperature, as well as other primary crystallization variables.

When crystals are not obtained in the initial screen, review droplets with precipitates for microcrystallinity. Examine the amorphous material under a high power microscope between crossed polarizing lenses to look for birefringence. True amorphous precipitates do not glow. Microcrystalline precipitates may glow under polarization. Streak seeding may be used in these situations towards differentiating microcrystals from precipitate as well as for producing larger, single crystals.

If the amorphous material is precipitate, consider one of the following:

a. Screen an alternate sample or precipitant concentration
b. Vary the drop ratio
c. Change the temperature of the experiment.

If the droplet remains clear, continue to observe the screen for several weeks and consider increasing sample concentration or increasing the concentration of the precipitant.

Using the unique dehydrant format one can simply increase the concentration of the dehydrant by adding concentrated dehydrant (100%) to the 24% w/v dehydrant to increase dehydrant concentration. Or one can simply replace the 24% w/v dehydrant with a more concentrated dehydrant such as 30 or 50% w/v PEG 3,350.

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If small crystals are grown which are not suitable for X-ray diffraction analysis there are several options to pursue.

a. Use the small crystals as seeds to grow larger crystals.
b. Set optimization trials, varying the primary crystallization variables to optimize conditions for crystal growth.

Review all of the results in the initial screen to obtain information on what affects pH, precipitant type and concentration, as well as the mixing of salts with precipitants have on crystal growth. Design subsequent trials to encompass these variables in a grid. If the results of the screen performed at room temperature do not appear different from the other temperatures, pursue varying pH, precipitant type and concentration, salt, cation and additive during optimization. If the presence or precipitate or crystals is dependent upon temperature, implement temperature variations into the crystallization strategy.

References and Readings

1. Harris, et al., Proteins: Structure, Function, and Genetics, (1995) 23, 285-289.

Technical Support

Inquiries regarding Low Ionic Strength 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.

Hampton Research
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Website: www.hamptonresearch.com

Tube #	Buffer	Tube #	Precipitant	(Recommended Dehydrant)
1.	0.05 M Potassium chloride pH 2.0	A.	4% w/v Polyethylene glycol 3,350	(24% w/v Polyethylene glycol 3,350[1])
2.	0.05 M Citric acid pH 3.0	B.	8% w/v Polyethylene glycol 3,350	
3.	0.05 M Citric acid pH 3.5	C.	12% w/v Polyethylene glycol 3,350	
4.	0.05 M Citric acid pH 4.0	D.	16% w/v Polyethylene glycol 3,350	
5.	0.05 M Citric acid pH 4.5	E.	20% w/v Polyethylene glycol 3,350	
6.	0.05 M Citric acid pH 5.0	F.	24% w/v Polyethylene glycol 3,350	
7.	0.05 M Citric acid pH 5.5			
8.	0.05 M MES monohydrate pH 6.0			
9.	0.05 M BIS-TRIS pH 6.5			
10.	0.05 M Imidazole pH 7.0			
11.	0.05 M HEPES pH 7.5			
12.	0.05 M Tris pH 8.0			
13.	0.05 M Tris pH 8.5			
14.	0.05 M Glycine pH 9.0			
15.	0.05 M Glycine pH 9.5			
16.	0.05 M Glycine pH 10.0			
17.	0.05 M Sodium phosphate dibasic pH 11.0			
18.	0.05 M Sodium phosphate dibasic pH 12.0			

1. Available separately

Not included with Low Ionic Strength Screen kit. Catalog number HR2-519 24% w/v Polyethylene glycol 3,350, 200 milliliters.

34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
e-mail: tech@hrmail.com
Website: www.hamptonresearch.com

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