January 2010

EasyXtal® and NeXtal® Protein Crystallization Handbook

EasyXtal 15-Well Tools

NeXtal Evolution μplate

NeXtal DWBlocks

NeXtal Tubes

EasyXtal Pre-Screen Assay

Opti-Salt Kit

For setup of protein crystallization trials

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

Prefilled NeXtal DWBlocks	
Catalog no.	**Varies**
NeXtal DWBlock	1 deep-well block filled with 96 x 1.5 ml Screening Suite solutions
Piercing Tool	1
Adhesive Foil	1 sheet
Screening Suite Composition Table	1

Prefilled NeXtal Tubes	
Catalog no.	**Varies**
NeXtal Tubes	96 x 10 ml tubes filled with Screening Suite solutions
Handbook	1
Screening Suite Composition Table	1

NeXtal Evolution *µ*plate	
Catalog no.	**132045**
NeXtal Evolution *µ*plate	10
Product Sheet	1

EasyXtal 15-Well Tools	**(20)**	**(100)**
Catalog no.	**132006**	**132007**
EasyXtal Crystallization Plates	20	100
Crystallization Supports	20 x 15	100 x 15
Product Sheet	1	1
Handbook	1	1

EasyXtal 15-Well Tools X-Seal	**(20)**	**(100)**
Catalog no.	**132008**	**132009**
EasyXtal Crystallization Plates	20	100
X-Seal Crystallization Supports	20 x 15	100 x 15
Product Sheet	1	1
Handbook	1	1

EasyXtal 15-Well DG-Tools	**(20)**	**(100)**
Catalog no.	**132106**	**132107**
EasyXtal Crystallization Plates	20	100
DropGuard Crystallization Supports	20 x 15	100 x 15
Product Sheet	1	1
Handbook	1	1

EasyXtal 15-Well DG-Tool X-Seal	**(20)**	**(100)**
Catalog no.	**132108**	**132109**
EasyXtal Crystallization Plates	20	100
DropGuard X-Seal Crystallization Supports	20 x 15	100 x 15
Product Sheet	1	1
Handbook	1	1

EasyXtal Pre-Screen Assay	
Catalog no.	**130222**
24-well DropGuard Crystallization Tool prefilled with screening solutions	1
DropGuard X-Seal Crystallization Supports	24
Handbook	1

NeXtal DWBlock Opti-Salt Suite	
Catalog no.	**130921**
Pierceable deep-well block containing 96 x 0.5 ml Opti-Salt Suite solution, piercing tool, and adhesive foil	1
Handbook	1

Storage

EasyXtal and NeXtal Screening Suites should be stored at room temperature (15–25°C). NeXtal Tubes and DWBlocks can be stored under these conditions for up to 9 months without any reduction in performance. The EasyXtal Pre-Screen Assay can be stored at room temperature (15–25°C) for up to 6 months without any reduction in performance.

Product Use Limitations

EasyXtal and NeXtal products are intended for molecular biology applications. These products are neither intended for the diagnosis, prevention, or treatment of a disease, nor have they been validated for such use either alone or in combination with other products. Therefore, the performance characteristics of the products for clinical use (i.e., diagnostic, prognostic, therapeutic, or blood banking) are unknown.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding EasyXtal and NeXtal products or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of EasyXtal and NeXtal products is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

Obtaining crystals for protein 3D structure determination requires screening of a wide array of crystallization conditions. Starting with different sets of initial screening conditions, the search for a suitable condition can be narrowed down by further developing conditions centered around a promising condition that provides crystalline forms (i.e., "a hit").

QIAGEN offers the world's largest range of screening conditions, systematically arranged into Screening Suites. Each Screening Suite consists of a set of 96 precisely defined chemical solutions. There are suites suitable for initial screenings (e.g., The Classics Suite), and suites used to analyze specific chemical effects (e.g., The pHClear Suite). Many factors may influence the crystallization of a protein, such as:

- Protein concentration and purity
- Precipitant concentration
- pH value
- Buffer composition
- Presence of salt ions
- Presence of additives (e.g., inhibitors or metal ions)

Optimization of protein using the EasyXtal Pre-Screen Assay

Before starting crystallization experiments, it is recommended that purity and homogeneity of each batch of protein is checked using SDS-PAGE, size exclusion chromatography, dynamic light scattering, or mass spectrometry (if available).

The initial concentration of the protein solution is an important factor in obtaining crystals. Typically, this concentration lies between 5 and 10 mg/ml. The traditional approach is to use one protein concentration for all precipitant types — salts, polymers, and organics. This does not take protein solubility into account, which usually varies according to precipitant type. Starting initial screening with a suboptimal protein concentration wastes protein and precious time.

The EasyXtal Pre-Screen Assay helps to optimize protein concentration for the different precipitant types and pH extremes, increasing the probability of nucleation and crystallization events during initial screenings.

The EasyXtal Pre-Screen Assay is arranged in three mini-grids (8 conditions each), with one mini-grid for each precipitant type. Using two concentrations of the same chemical, a direct correlation between protein concentration, precipitant concentration, and precipitant chemical family can be observed.

When the protein concentration for a given precipitant type is optimal, a clear drop should be obtained in the well with low precipitant concentration, and a precipitated drop in the well with high protein concentration (Figures 1 and 2). The results of the assay can be used to establish the optimal balance between protein and precipitant concentrations required for crystallization.

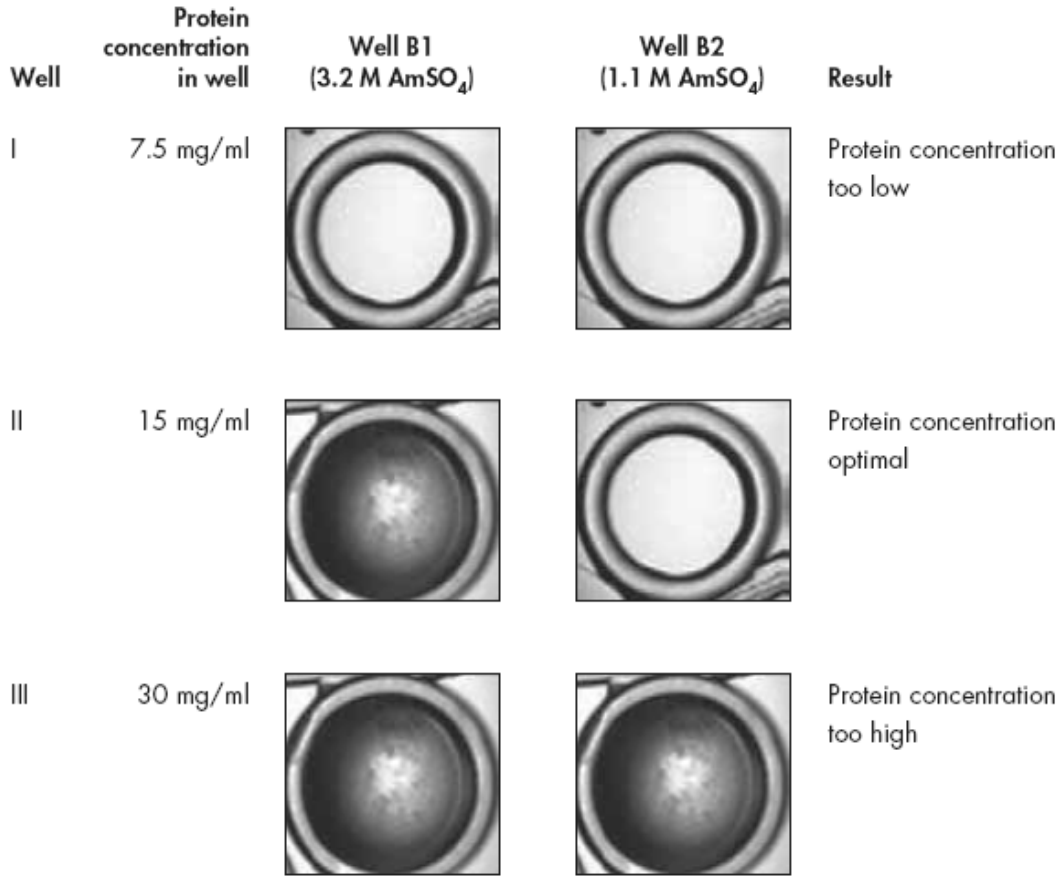


Figure 1. Optimization of protein concentration. The EasyXtal Pre-Screen Assay identifies the optimal protein concentration for crystallization. Pairs of crystallization supports containing 3 drops of protein at different concentrations are screwed into position above wells containing a high and low concentration of a precipitant. The optimal protein concentration for a given precipitant is one which shows a precipitate at the high precipitant concentration and a clear drop at the low precipitant concentration.

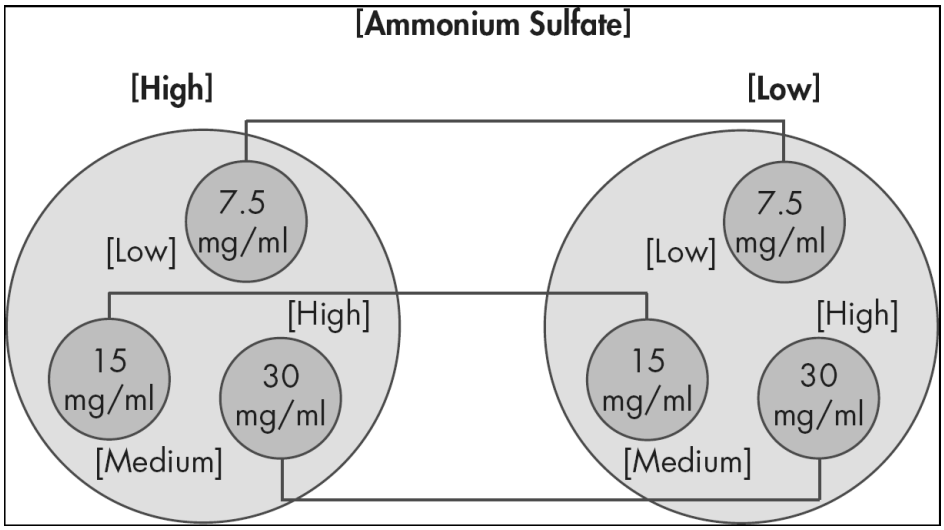


Figure 2. Comparison scheme for a set chemical pair using high, medium, and low protein concentrations on DropGuard supports.

Crystallization screening setup

Vapor diffusion is the most widely used method for crystal growth. It can be performed using a sitting drop (e.g., using the NeXtal Evolution μplate) or a hanging drop (e.g., using the EasyXtal 15-Well Tool) method (Figure 3). Both methods have been shown to successfully yield crystals.

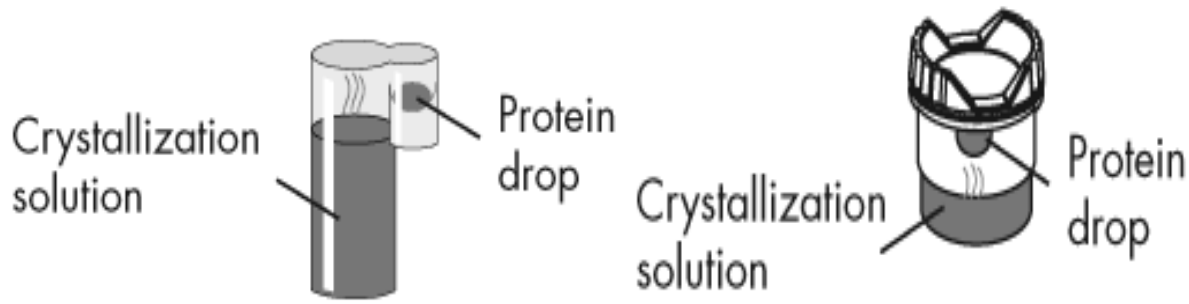


Figure 3. Vapor diffusion experiment. A Sitting drop versus B hanging drop setup.

For initial screening, setup in 96-well format is most effective because the amounts of protein and screening solution required are minimized. The NeXtal Evolution μplate allows for manual and automated setup of drops in the microliter and nanoliter range.

The crystallization phase diagram

The crystallization event can be best described using the phase diagram. A phase diagram describes the behavior of a mix of protein and precipitant at different concentrations (Figure 4). For example, at low concentrations of both protein and precipitant, the drop remains clear (condition 1 in Figure 4). As the concentration of both partners increases during the vapor diffusion experiment, they pass through the metastable phase to the nucleation phase, where first tiny crystals can form (condition 2 in Figure 4). As these crystals grow, they use up protein, thereby decreasing protein concentration and reverting back to the metastable phase, where no new crystals are nucleated, but the existing small crystals grow larger (condition 3 in Figure 4). If the concentration of protein and precipitant is too high, precipitates form immediately without forming crystals (condition 4 in Figure 4). Evaporation from the vapor diffusion vessel has a considerable effect because it pushes the drop to the precipitation phase (condition 4 in Figure 4). This effect is usually uncontrollable and difficult to reproduce. The extent of evaporation depends on the composition of the screening solution, as well as on the plastic and sealing equipment used.

Reproducibly low evaporation can be achieved with the NeXtal Evolution μplate (Figures 5 and 6). Its broad rims enable a tight interaction with the sealing tape, and even after individual wells have been cut open to harvest a crystal, the remaining wells can be incubated further (Figure 5).

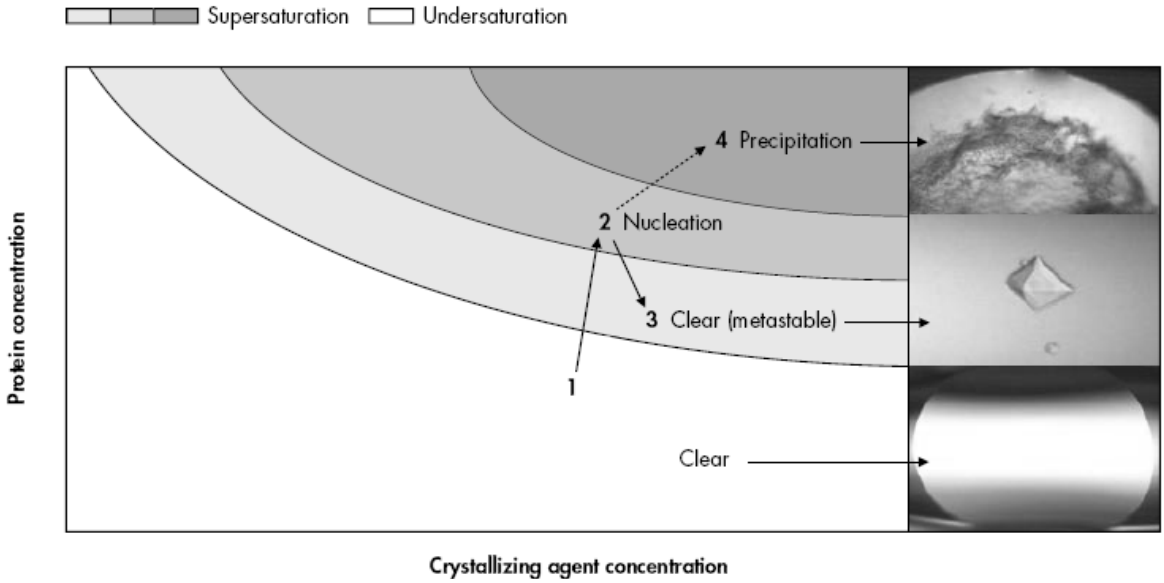


Figure 4. The crystallization phase diagram.

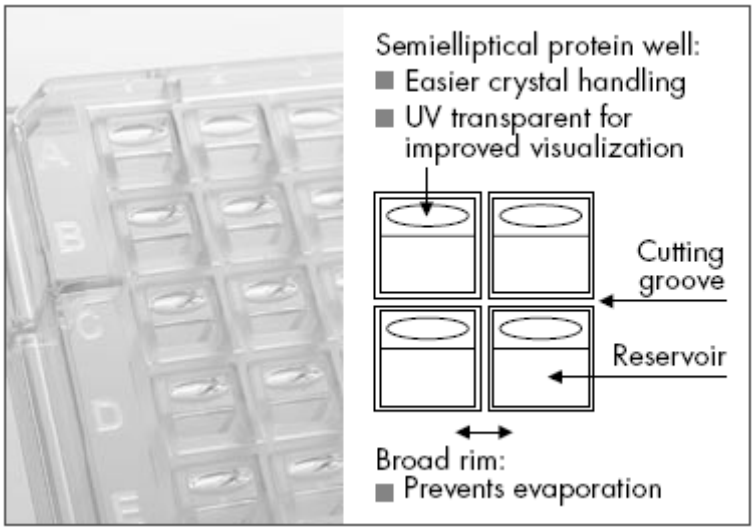


Figure 5. The NeXtal Evolution *µ*plate and important technical features.

Initial screening

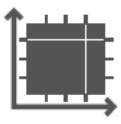
When starting a crystallization experiment, screening conditions should cover a large part of the chemical space to enhance crystal formation. Crystallization suites are usually either based on experimental conditions outlined in literature references and databases or based on a rational combination of chemical conditions. Although past experience can be used to deduce starting conditions for crystallization setup, it does not guarantee success.

Screening strategies and precipitant types

The general function of precipitants in protein crystallization experiments is to decrease the solubility of the protein. Polymers such as PEG, salts, and organic solvents are the most popular precipitants. There are 3 main screening strategies, as discussed below.

Sparse matrix

Sparse matrix (or incomplete factorial) strategies typically map a wide sector of the chemical space using many different reagents. Sparse matrix screens are ideally suited for first screening trials.



Grid screens

Grid screens systematically evaluate two factors, such as a precipitant, in different concentrations at various pH values. Usually, grid screens are used for refinement of successful conditions.

Ionic sampling

Ionic sampling uses the effect the ionic strength of a solution has on a

macromolecule (i.e., affecting the surface charges and therefore its solubility). This strategy keeps one component (e.g., pH), constant while varying a second component (e.g., different salts). An ionic sampling screen ensures further refinement of successful conditions.

Choice of crystallization screens

Even though screening thousands of different conditions increases the chances for growing crystals, the amount of protein available for a given crystallization experiment is usually the limiting factor. Choosing the right conditions for an initial screening is therefore a challenge. The Joint Center for Structural Genomics (JCSG) has statistically evaluated all commercially available screening solutions in more than half a million experiments (4). The result of their work is comprised in the JCSG Core Screens, which together form 384 unique screening conditions in a sparse matrix screen. If enough protein is available to screen 500 conditions, we recommend combining the JCSG Core I–IV Suites with the PACT Screen (6) for initial experiments. If only 200 conditions can be screened, the JCSG+ Suite from the same group in combination with the PACT Suite has been shown to be effective (7). All screening suites are available as 10 ml tubes and as automatable 1.5 ml deep-well (DW) blocks. Table 1 on page 15 summarizes the screens recommended for initial screening.

Initial screening experiments should be performed in a temperature-controlled and vibration-free environment. A typical strategy is to set up duplicate screens at different temperatures (e.g., 4°C and 16°C). Moving the screening plates unnecessarily should be avoided.

After setup, plates should be inspected under a stereo-microscope at regular time intervals (e.g., after 1 day, 2 days, 5 days, and 10 days). After 24 hours, the presence of precipitate in ~50% of the 96 conditions indicates that the protein concentration is correct. Observations can be recorded using scoring sheets, which can be downloaded at www.qiagen.com/EasyXtal.

Table 1. Screening suites from QIAGEN

Description	Suite
Core screens for initial investigations	
Statistically evaluated solutions	JCSG Core Suites (I–IV)*
Systematic effect of pH, anions, and cations	PACT Suite*
Using an optimized sparse matrix	JCSG+ Suite
Using classic solutions	Classics, Classics II, Classics Lite, and Cryos Suites
Rapid analysis of polymers, alcohols, and salts	ComPAS Suite
Screens analyzing single precipitant types	
Varying salts and pH	Anions and Cations Suites and the pHClear and pHClear II Suites
Using polyethylene glycols of varying molecular weights	PEGs and PEGs II Suites
Using ammonium sulfate	AmSO4 Suite
Using 2-methyl-2,4-pentanediol (MPD)	MPD Suite
Screening conditions for specialized applications	
For membrane proteins in cubic phase or sponge phase experiments	CubicPhase I and CubicPhase II Suites†
For membrane proteins in classical vapor diffusion experiments	MbClass and MbClass II Suites
For protein–protein complexes	Protein Complex Suite
For protein–nucleic acid complexes	Nucleix Suite
Rapid optimization of initial crystallization hits	
Salt additives at different concentrations and pH	Opti-Salts Suite

* Recommended for a standard initial screen of 500 conditions.

† To be used with NeXtal CubicPhase μplates.

For detailed information on individual condition compositions, please visit www.qiagen.com/protein/crystallization.

Interpretation of results

While the setup of a vapor diffusion experiment is straightforward, interpretation of results requires more expertise. The following section provides typical examples of the most commonly observed results.

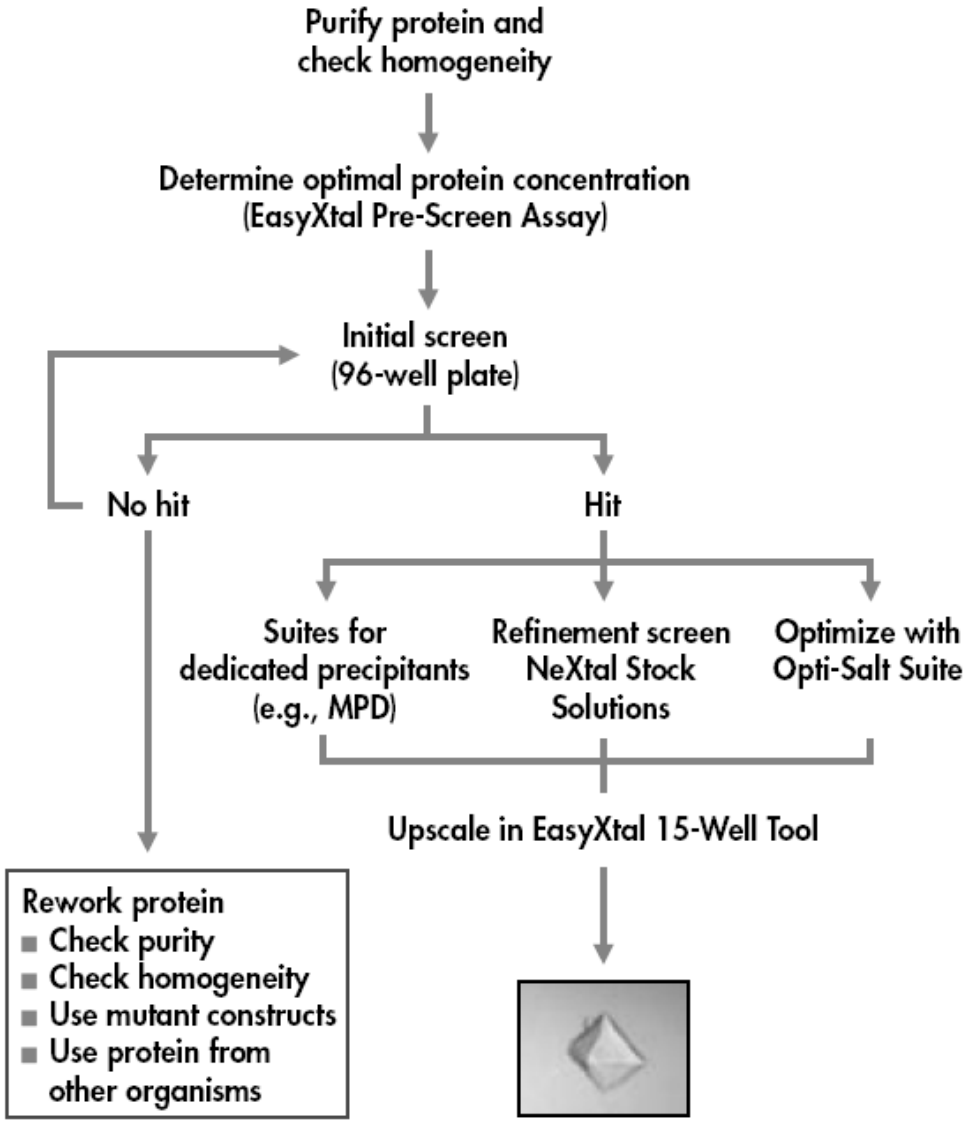


Figure 6. Decision tree for optimization experiments. Several approaches can be taken when a hit condition is identified and these can be performed in conjunction with each other or separately.

Refinement and optimization

Several approaches can be taken in order to fine-tune successful hit conditions and to grow larger and/or better-diffracting crystals (Figure 6). One strategy involves choosing suites using chemicals that worked best in the initial screening for a second, more defined round of screening using dedicated precipitants. Another easy optimization strategy involves use of the Opti-Salts Suite, which

comprises of premixed salt additive solutions at different pH available in deep-well blocks. The 96 conditions from this suite are mixed with the original hit condition (available separately as an EasyXtal Refill-Hit) to yield 96 new conditions that form an optimization grid around the originally successful condition (Figure 7). This method has been successful in many cases and can be performed in 96-well format.

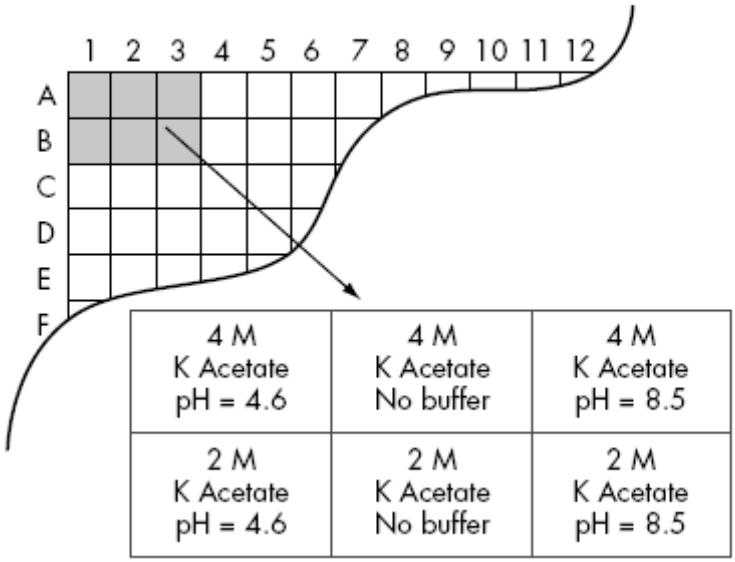


Figure 7. Principle of Opti-Salt optimization grids. The method involves screening of 16 different chemicals in grids of 6, where concentration of the additive and pH are varied compared to the original hit condition. Optimization conditions can also be prepared from NeXtal Stock Solutions that contain exactly the same chemicals as the original screening solutions.

Optimization conditions can also be prepared using QIAGEN's NeXtal Stock Solutions that contain exactly the same chemicals as the original screening solutions. This optimization step can be performed in a 96-well format, but usually a larger format is used to limit the number of solutions to be prepared and to grow larger crystals. The EasyXtal 15-Well Tool is highly suited for optimization experiments because the original hit can be placed in the center well as a control and the variation grid can be set up around the central position.

Upscaling

In cases where the crystals grown in small drops in 96-well format are not large enough for data collection, upscaling to a larger drop size can be attempted. The EasyXtal 15-Well Tool is highly suitable for setting up drops in the microliter range. Converting an experiment from smaller to larger volumes has an impact on the surface-to-volume ratio, and further optimization is often required to achieve similar results to those obtained in the nanoliter range.

The EasyXtal 15-Well Tool also allows for a number of other techniques. These include:

- Screening of cryoprotectants
- Screening of ligands
- Seeding experiments

EasyXtal Tools are available with standard and DropGuard (DG) crystallization supports (Figure 8). The DropGuard crystallization support accommodates 3 drops of 2 µl total volume (1 µl protein + 1 µl precipitant) or 3 drops of a 4 µl total volume. Wells are numbered for easy identification, drop spreading is prevented, and visualization of the drops is enhanced (Figure 9). X-Seal crystallization supports contain X-Seal, which is made from an advanced O-ring material that minimizes evaporation and prolongs the reuse of reservoir solutions.

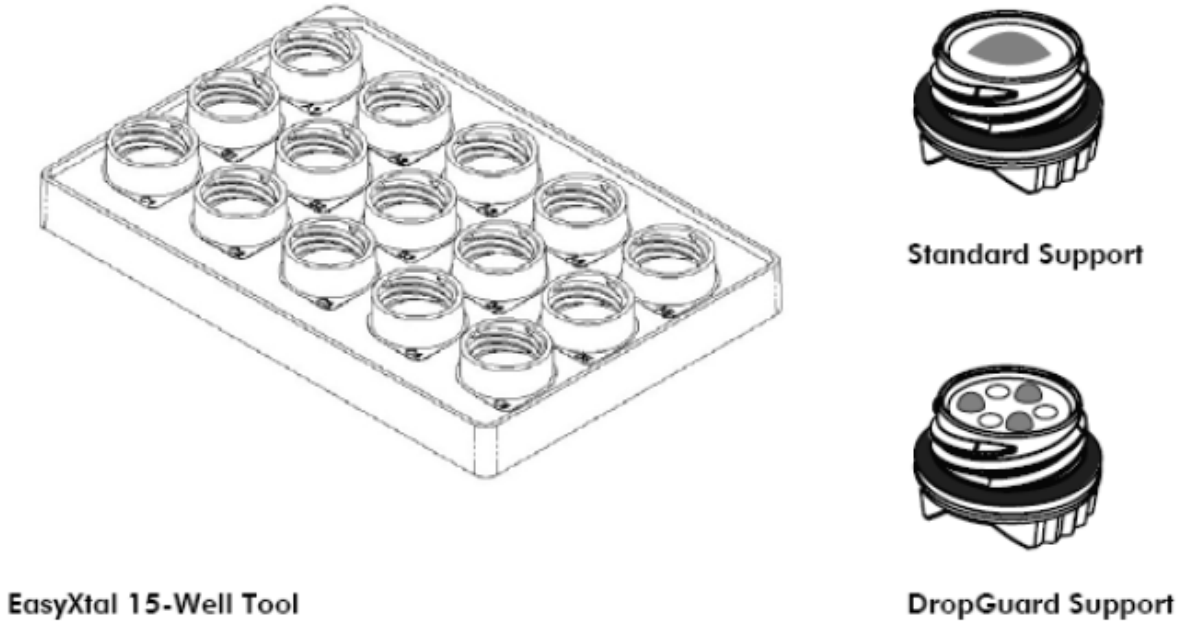


Figure 8. EasyXtal15-Well Tool and crystallization supports.

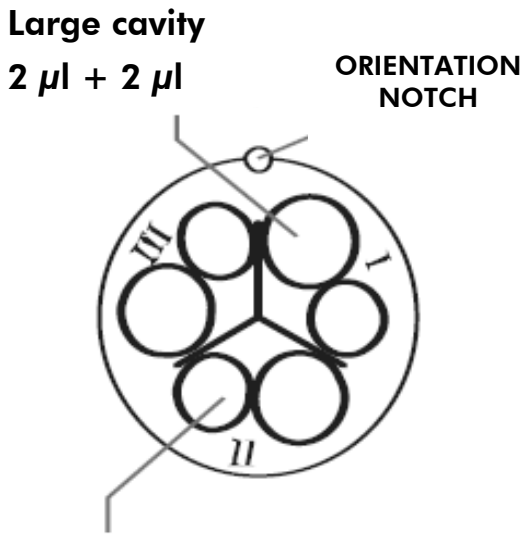


Figure 9. The EasyXtal 15-Well Tool DropGuard crystallization support design.

Please refer to the appendices for a range of other special crystallization techniques that can be performed with the EasyXtal 15-Well Tool.

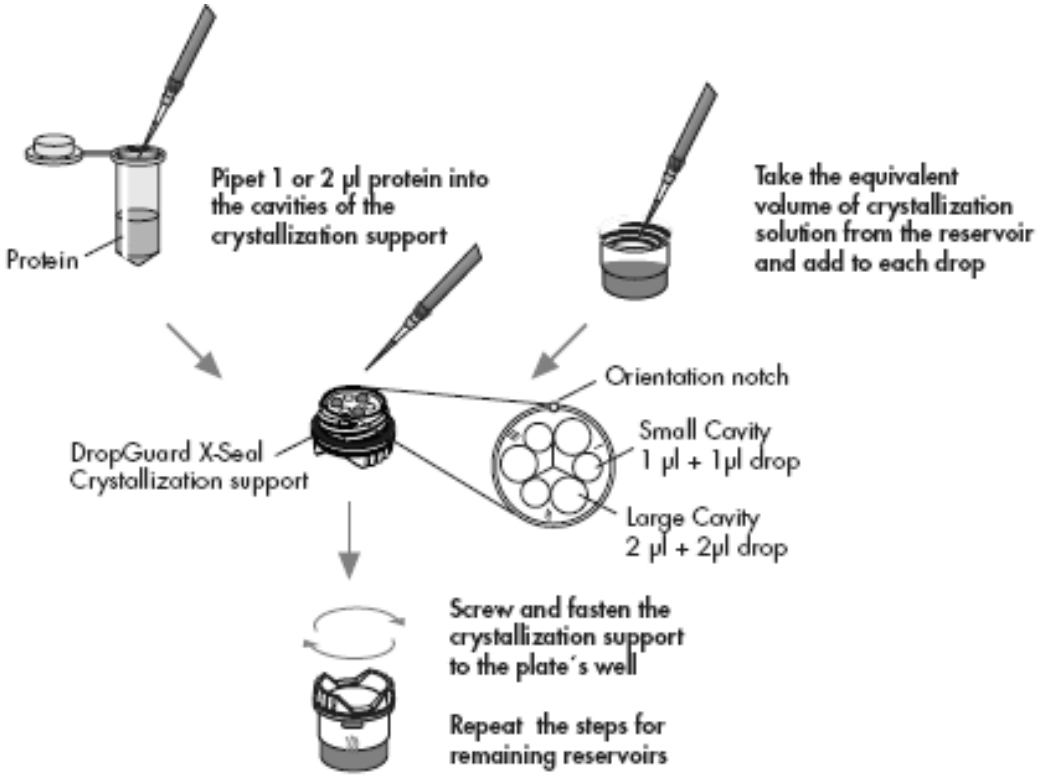


Figure 10. Setting up crystallization trials using DropGuard crystallization supports.

Protocol: Setting up a Pre-Screen Assay Experiment

The pre-screen assay helps to optimize protein concentrations for the different precipitant types and pH extremes increasing the probability of nucleation and crystallization events during initial screenings.

Reagents to be supplied by user

- Protein solution

Procedure

1. **Prepare dilutions of the protein solution. This results in 3 solutions (e.g., 5, 10, 20 mg/ml) with different concentrations.**
2. **Use the cap remover tool supplied with the kit to remove the transportation cap from a well of one of the crystallization plates.**
3. **Pipet 1 µl (small cavity) or 2 µl (large cavity) of each diluted protein solution into the three wells of a crystallization DG-support. Follow the numbering (i.e., always pipet the lowest protein concentration into well I, etc).**

   Optional: Mix the protein and the Screening Suite solution carefully but thoroughly. Some researchers prefer to simply pipet both drops together without mixing.
4. **Carefully screw the crystallization support into place above the well from which the Screening Suite solution was taken in step 3.**

   IMPORTANT: Do not overtighten.
5. **Repeat steps 2–4 for all remaining wells.**

Protocol: Setting up Crystallization Trials Using NeXtal DWBlocks and NeXtal Evolution µplates

NeXtal DWBlocks are pierceable deep-well blocks containing 96 x 1.5 ml Screening Suite solutions per well. They are supplied with a piercing tool and adhesive foil. The solutions can be used for manual or automated setup of crystallization screenings in 96-well microplates.

We recommend the NeXtal Evolution µplate for setup because unwanted evaporation of solution from the plate is minimized. Its broad rims allow for a tight interaction with the sealing tape, and even after individual wells have been cut open to harvest a crystal, the remaining wells can be incubated further. The single well allows for easy drop setup and manipulation of drops during crystal harvesting. Furthermore, the plate is highly transparent for visible, polarized, and UV light.

Equipment and reagents to be supplied by user

- Protein solution
- EasyXtal Sealing Tapes (cat no. 132105)
- NeXtal Evolution µplate (cat. no. 132045 or 132046)

Procedure

1. **Use the piercing tool to pierce the seal of the NeXtal DWBlock and access the Screening Suite solutions.**
2. **Use an automated liquid-handling station or multichannel pipettor to pipet solutions (typically 50–70 µl) into the solution reservoirs of a NeXtal Evolution µplate.**
3. **Once the desired volume has been removed, reseal the NeXtal DWBlocks using the adhesive foil.**
4. **Transfer the desired volume (0.1–1 µl) of protein into each protein well of the NeXtal Evolution µplate.**

   Note: We recommend using a multichannel pipettor or robot for protein transfer. Work quickly to minimize evaporation.
5. **Transfer an equivalent volume of Screening Suite solution from the respective well into the protein wells.**

   Optional: Mix the protein and the Screening Suite solution carefully but thoroughly. Some researchers prefer to simply pipet both drops together without mixing.
6. **Immediately seal the NeXtal Evolution µplate with sealing tape.**

Protocol: Setting up Crystallization Trials Using EasyXtal 15-Well Tools and EasyXtal 15-Well Tools X-Seal

NeXtal Tubes offer Screening Suite solutions in a bulk format (10 ml tubes). The solutions can be used for manual or automated setup of crystallization experiments in 15-well plates or 96-well microplates. EasyXtal 15-Well Tools consist of a 15-well plate and 15 EasyXtal DropGuard crystallization supports. They are available as standard and X-Seal supports. Standard crystallization supports have a white O-ring which is suitable for most crystallization solutions. X-Seal crystallization supports contain the X-Seal, which is made from an advanced O-ring material that minimizes evaporation and prolongs the reuse of reservoir solutions. As more and more laboratories work with a wider range of different, sometimes volatile chemicals, X-Seal crystallization supports enable users to set up conditions without having to worry about evaporation.

Equipment and reagents to be supplied by user

- Protein solution
- NeXtal Tube prefilled with Screening Solution or optimizing solution prepared from NeXtal Stock Solutions

Procedure

1. **Open the NeXtal Tubes and transfer a suitable volume of screening solution (500–700 µl) to the reservoirs of an EasyXtal 15-Well Tool.**
2. **Pipet 1–5 µl of protein solution onto the center of a crystallization support.**
3. **Pipet an equivalent volume of Screening Suite solution from the well into the protein drop.**

   Optional: Mix the protein and the Screening Suite solution carefully but thoroughly. Some researchers prefer to simply pipet both drops together without mixing.
4. **Carefully screw the crystallization support into place above the well from which the Screening Suite solution was taken in step 3.**

   IMPORANT: Do not overtighten.
5. **Repeat steps 2–4 for all remaining wells.**

   Note: The screw-in supports allow easy opening and closing of wells to modify conditions without having to set up a new tray. By diluting or increasing the concentrations of the Screening Suite solution, it may be possible to transform precipitate or clear drops into crystals. Please see the appendices for further information.

Protocol: Setting up Crystallization Trials Using NeXtal Tubes in EasyXtal DG-Tools and EasyXtal DG-Tools X-Seal

NeXtal Tubes offer Screening Suite solutions in a bulk format (10 ml tubes). The solutions can be used for manual or automated setup of crystallization experiments in 15-well plates or 96-well microplates.

EasyXtal DropGuard crystallization supports enable easy setup of multiple drops per well and eliminate problems such as drop spreading while using organic chemicals such as MPD or detergents. Each support contains three cavities to accommodate 1 µl + 1 µl drops and three others for 2 µl + 2 µl drops. The cavities isolate drops and prevent contact between them, enabling easy recovery of crystals without disrupting neighboring drops. DropGuard crystallization supports enable testing of different protein concentrations or screening of different ligands or additives in the same well.

X-Seal supports contain X-Seal, which is made from an advanced O-ring material that minimizes evaporation and prolongs the reuse of reservoir solutions. As more and more laboratories work with a wider range of different, sometimes volatile chemicals, X-Seal crystallization supports enable users to set up conditions without having to worry about evaporation.

Equipment and reagents to be supplied by user

- Protein solution
- NeXtal Tube prefilled with Screening Solution or optimizing solution prepared from NeXtal Stock Solutions

Procedure

1. **Open the NeXtal Tubes and transfer a suitable volume of screening solution (500–700 µl) to the reservoirs of an EasyXtal 15-Well Tool.**
2. **Pipet up to 1 µl (small cavity) or 2 µl (large cavity) of protein solution into a DropGuard or DropGuard X-Seal crystallization support.**
3. **Pipet the same volume of Screening Suite solution from the reservoir into the protein drops.**

   Optional: Mix the protein and the Screening Suite solution carefully but thoroughly. Some researchers prefer to simply pipet both drops together without mixing.
4. **Carefully screw the crystallization support into place above the well from which the Screening Suite solution was taken in step 3.**

   IMPORTANT: Do not overtighten.
5. **Repeat steps 2–4 for all remaining wells.**

Note: The screw-in supports allow easy opening and closing of wells to modify conditions without having to set up a new tray. By diluting or increasing the concentrations of the Screening Suite solution, it may be possible to transform precipitate or clear drops into crystals. Please see the appendices for further information.

Protocol: Optimizing Crystallization Conditions Using the Opti-Salts Suite

To avoid the time-consuming process of developing expanded grids around an original hit condition, the Opti-Salts Suite offers an easy-to-use kit for crystallization optimization. Organized in 15 mini-grids (6 conditions each) and 1 control mini-grid, the Opti-Salts Suite facilitates easy identification of the most important variables. Individual Screening Suite conditions are available as Refill-Hit Solutions (4 x 12.5 ml tubes). By mixing the original hit condition in a ratio of 9:1 with the Opti-Salts solutions, a new and unique subset of crystallization conditions is generated. Mixing can be either performed manually or using an automated liquid-handling system.

Equipment and reagents to be supplied by user

- EasyXtal Refill-Hit Solution (a total of 5 ml is required per microplate)
- EasyXtal Sealing Tapes (100) (cat. no. 132105)
- NeXtal Evolution µplate (cat. no. 132045 or 132046)
- Protein solution

Procedure

1. **Transfer 5 µl of the EasyXtal Refill-Hit solution to each reservoir of a NeXtal Evolution µplate.**
2. **Use the piercing tool to pierce the seal of the NeXtal DWBlock and access the Opti-Salt Suite solutions.**
3. **Use an automated liquid-handling station or multichannel pipettor to pipet 45 µl of Opti-Salt Solution into the solution reservoirs of the NeXtal Evolution µplate.**
4. **Reseal the NeXtal DWBlock using the adhesive foil.**
5. **Mix solutions in the reservoir by pipetting up and down.**
6. **Transfer 0.1–1 µl protein into each protein well of the NeXtal Evolution µplate.**

   Note: We recommend using a multichannel pipettor or robot for protein transfer.
7. **Transfer an equivalent volume of Screening Suite solution from the respective well into the protein wells.**

   Optional: Mix the protein and the Screening Suite solution carefully but thoroughly. Some researchers prefer to simply pipet both drops together without mixing.
8. **Repeat steps 6 and 7 for all wells.**
9. **Immediately seal the NeXtal Evolution µplate with the sealing tape.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Condensation and cross-contamination problems	
Leakage of NeXtal DWBlocks	To avoid leakage and evaporation, NeXtal DWBlocks are heat-sealed using a pierceable/peelable aluminum foil. To access solutions, the foil is simply pierced with the supplied piercing tool. This process reduces condensation and cross-contamination problems. NeXtal DWBlocks can be repeatedly resealed using adhesive aluminum foils (provided with each NeXtal DWBlock).
Drops spread in EasyXtal crystallization supports	
Protein buffer and the crystallization condition	Use EasyXtal 15-Well DG-Tools, which contain DropGuard crystallization supports. These supports contain 6 cavities and have been designed to prevent drops from spreading when using conditions containing organic solvents (e.g., MPD) or detergents.

	Comments and suggestions
Formation of salt crystals in protein drop	
Concentration of precipitant solution too high	Reduce concentration of precipitant. Use a microscope equipped with a UV light source to establish if they are protein or salt crystals. Proteins can show epifluorescence at 340 nm due to tryptophan residues which can be detected. However, if the protein does not contain any tryptophan residues, or if fluorescence quenching occurs, this method cannot be applied. Alternatively, the absorption of aromatic residues at 280 nm (which is also commonly used for protein quantification) can be used for visualization of protein crystals. Use the NeXtal Evolution μplate as it is highly UV transparent.
Formation of a clear drop	
a) Initial concentrations of protein and/or precipitate too low	Increase the concentration of protein and/or precipitant in the next round of screening.
b) Incubation temperature too high	Decrease the incubation temperature.
A precipitate is obtained instead of crystals	
Protein and/or precipitant concentrations are too high for crystal formation	There are two types of precipitates — those that consist of denatured protein and those that contain native protein and can be resolubilized. See Appendix A (page 29). Conditions that result in the denaturation of protein should be avoided in the future. If the protein remains in its native conformation, protein and/or precipitant concentrations can simply be decreased.
Phase separation occurs	
Formation of oily areas that form in the drop	These areas have increased protein concentration. This state favors crystallization, however, further optimization may be required. In parallel to optimization, the original drop should be further incubated because it may lead to crystal growth over time.

	Comments and suggestions
Formation of microcrystals and needles	
a) Concentration of the protein and/or precipitant is slightly too high and the experiment is in the nucleation phase	Decrease the concentration of protein and/or precipitant or seed into a fresh drop of protein solution. Alternatively, set up the experiment at a lower temperature, as this may lead to larger crystal growth due to altered growth kinetics.
b) Slow down in nucleation/crystal growth	Overlay the reservoir solution with an evaporation barrier of silicone oil, paraffin oil, or a mixture of both oils to slow down the vapor diffusion experiment or reduce the protein concentration.
No hit conditions are obtained even after evaluating many crystallization experiments	
Several reasons	Check purity and homogeneity of the protein. Variations of the protein should be generated. For example, these modifications can involve removal of flexible loops, reduction of protein surface charge, or expression and purification of only subdomains of the protein. Alternatively, homolog proteins from other organisms can be tested.

Appendix A: Pro-active Strategy

If a precipitate is obtained, it usually means that the protein and/or precipitant concentrations are too high for crystals to form. There are 2 types of precipitates — those that consist of denatured protein and those that contain native protein and can be resolubilized. If the protein becomes denatured, those conditions should be avoided in the future. If the protein stays native, protein and/or precipitant concentrations should be reduced.

Procedure

A1. Set up the condition again in an EasyXtal 15-Well Tool.

A2. Dilute the reservoir solution or replace with water.

A3. Incubate the experiment further.

> **Note:** If the protein is native, it will go back to a clear drop after some time. If not, it is irreversibly damaged.

Appendix B: Drug/Ligand Screening

Determination of protein structures complexed with drugs/ligands is of great importance in the study of cellular mechanisms, and allows observation of the interactions that are involved in the binding of drug/ligand to the protein and the overall effect on protein structure (1). It also indicates where the drug/ligand can be chemically modified and takes advantage of additional features at the binding site in the process of structure-based rational drug design (2). One method for producing protein crystals in which the drug/ligand is complexed to the protein requires screening for experimental conditions where the protein crystals are incubated in presence of drug/ligand solutions.

EasyXtal crystallization supports enable wells to be opened and closed as often as desired and eliminate the need to transfer crystals to depression wells, avoiding the risk of losing crystals during manipulation. The addition of the drug/ligand solution to the drop, and the removal of crystals from the drop are simple and straightforward procedures that do not disturb the remaining crystals. There is less chance of losing the complexed crystals due to drying up of drops during manipulation, or because of missing or losing a successful condition.

Procedure

B1. Prepare crystals in an EasyXtal 15-Well Tool.

> **Note:** Try to optimize conditions such that 6–8 crystals are obtained in 5–10 µl drops. Prepare as many drops as drugs/ligands that need to be screened.

Note: Prepare drug/ligand solutions at a 10x concentration (preferably in a buffer that is similar to the Screening Suite solution of the drops).

B2. Open the well containing crystals and pipet 0.5–1 µl of the concentrated drug/ligand solution into the equilibrated drops (volume of drug/ligand solution used should be 1/10 volume of the drop).

B3. Close the well.

B4. Record the time and observe crystals under the microscope. Ensure that the crystals do not deteriorate.

B5. Open the well after 2 h, 4 h, 8 h, and 12 h, and scoop up a single crystal. Reclose the well and freeze the crystal using cryoprotectant.

B6. Collect X-ray diffraction data and verify the presence of drug/ligand in structure.

Appendix C: Crystallization Using the Same Well

In the process of structural studies of macromolecules by x-ray crystallography, additional crystals are often required to solve a protein's structure. One may also want to work with a protein preparation that differs slightly from the native protein (e.g., mutant enzymes, which are often needed for the determination of enzymatic reaction mechanisms [3]).

For example, the use of selenomethionine (Se-Met)-labeled protein for phase determination (which is now routine in structural biology laboratories) requires the growth of crystals from a selenomethionine-enriched protein preparation (4). In such cases, returning to the initially successful crystallization well is a good starting point for growth strategies.

Procedure

C1. Obtain diffraction-quality crystals of your native protein.

C2. Prepare Se-Met–enriched protein or mutant enzymes.

C3. Open the well that contains the crystallization support with native crystals. On the same crystallization support, prepare a drop containing Se-Met–labeled/mutant protein.

C4. Record the position of the drops.

C5. Screw the crystallization support back into place.

C6. Collect X-ray diffraction data and verify the presence of drug/ligand in the structure. Alternatively, prepare a drop containing Se-Met–labeled/mutant protein on a new crystallization support and exchange for the support containing native crystals over the original well.

Appendix D: Heavy Atom Screening/Derivatization of Protein

In determining protein structures with X-ray crystallography, successful initial phasing of a completely new protein structure requires the incorporation of atoms with a high electron density in the protein crystal (5). Derivatization of proteins with heavy atoms is a commonly used technique to fulfill this requirement (6).

The heavy atom protein derivatives are prepared by introducing heavy atoms such as gold, lead, mercury, platinum, silver, or uranium at a few specific positions in the protein, without otherwise affecting either the protein crystal or protein structure. Experimental procedures to produce protein crystals in which heavy atoms are incorporated require screening for conditions where protein crystals are incubated in the presence of different heavy atoms. Since heavy atom solutions often damage protein crystals over time, it is also highly recommended to screen for time intervals that are compatible with crystal integrity (5).

Using EasyXtal crystallization supports, the addition of heavy atom solutions to the drop and the removal of crystals from the drop are simple and straightforward procedures that do not disturb the remaining crystals. The ability to open and close the well as many times as desired eliminates the need for transferring crystals to depression wells and the time required to find a "stabilizing" solution. Interval freezing of each of the crystals is made possible using this system.

Procedure

D1. Prepare crystals while trying to optimize conditions such that 6–8 crystals are obtained in 5–10 µl drops.

Note: Prepare as many drops as the number of heavy atoms* you want to screen.

IMPORTANT: Heavy atom solutions are toxic. Use gloves and a mask and work in a fume hood.

D2. Open the well containing the crystals and pipet 0.5–1 µl of the heavy atom solution into the 5–10 µl equilibrated drops (volume of heavy atom solution used is 1/10 volume of drop).

D3. Close the well.

D4. Record the time of addition and observe the crystals under a microscope. Ensure that the crystals do not deteriorate.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

D5. After an interval of 30 min, 1 h, 2 h, 4 h, 8 h, and 12 h, open the well and remove a single crystal. Close the well and freeze the crystal using cryoprotectant.

D6. Collect X-ray diffraction data and verify heavy atom derivation.

Appendix E: Streak Seeding

When working on crystallization of macromolecules, often large numbers of small nuclei are obtained from screening conditions; however, growing a large crystal is difficult. It is known that spontaneous nucleation often occurs at high degrees of supersaturation, thus favoring production of numerous small crystals or aggregates that do not necessarily render possible the crystal growth step. Because conditions for nucleation and crystal growth differ, optimizing these processes separately increases the probability of obtaining diffraction-quality protein crystals. This can be accomplished by transferring the nuclei from nucleation conditions to crystal-growth–optimized conditions. Streak seeding is a technique where small nuclei (or microcrystals) are transferred with the help of a seeding tool to a protein solution that is optimized for crystal growth. The following protocol is adapted from literature (7).

Note: Greaseless, screw-in EasyXtal crystallization supports allow users to work rapidly and facilitate the use of hanging drops in streak seeding experiments. Independently sealed crystallization supports allow users to repeat the experiment several times and facilitate pre-equilibration of hanging drops before streaking and access to the different drops at anytime during the experimental procedure.

Procedure

E1. Add protein to the Screening Suite condition that provides microcrystals (seed source well).

E2. In another well, prepare drops of the protein mixed with Screening Suite solution that is optimized for crystal growth (crystal growth well) and allow to equilibrate for 24–48 h.

E3. Open the seed source well and gently draw a seeding tool (e.g., Hampton Research, cat. no. HR8-133) through the drop to pick up seeds.

E4. Close the seed source well with the crystallization support.

Note: Work rapidly to avoid evaporation of the drops.

E5. Observe drops under a microscope to evaluate seeding efficiency.

Note: It should be possible to see crystals growing along the streak line.

E6. Repeat the experiment by varying protein concentration in the crystal growth well drops until you obtain the desired result.

Note: When using the seeding technique, it is recommended that only the protein concentration is varied (e.g., using 80%, 60%, and 40% of initial concentration) instead of the precipitant concentration to avoid melting of seed crystals in the drop.

The screening tools can be rinsed with 20% ethanol and reused.

If streak seeding does not produce the desired results, other techniques, such as micro- or macro-seeding and/or microdialysis, may prove useful.

Appendix F: Separating Nucleation from Crystal Growth

Determination of crystal structures requires diffraction quality protein crystals. Separating nucleation from crystal growth permits the optimization of these two different processes in order to obtain fewer but larger protein crystals. The most commonly used technique to perform such experiments is seeding. Another possibility is to control protein and precipitant concentrations remotely through vapor diffusion. Once nucleation has occurred, this method allows "dilution" of drops to provide conditions optimized for crystal growth. The following protocol is an adaptation of Saridakis and Chayen (2000) (8).

Note: Greaseless, screw-in EasyXtal crystallization supports make transfer from one condition to another simple. There is no chance of breaking cover slides or losing drops in the grease ring or oil. It is even possible to go back to the nucleation condition if no results appear in the metastable condition after the expected time interval.

Procedure

F1. Prepare hanging drops optimized for nucleation of the selected protein (nuclei source).

Note: Prepare as many drops as the number of time intervals to be evaluated.

F2. In corresponding wells, pipet optimized crystal growth solution (metastable condition) and cover with an empty crystallization support to avoid evaporation.

F3. Select time intervals within which crystal nuclei form.

F4. At each time interval, transfer the crystallization support from the nuclei source well to its corresponding optimized crystal growth well.

F5. Observe results under a microscope.

Note: Nucleation conditions usually promote production of several small crystals.

Time intervals can vary from minutes to several days, depending on the protein and the type of solution used (9).

Once a promising crystal growth condition has been identified, crystal quality can be further optimized by controlling the rate of vapor diffusion in the selected well.

One technique to slow down the crystallization process is to use an oil layer in vapor diffusion experiments, thus helping obtain fewer but better quality crystals (10).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Cited references

1. Saridakis, V., Christendat, D., Kimber, M.S., Dharamsi, A., Edwards, A.M., and Pai, E.F. (2001) Insights into ligand binding and catalysis of a central step in NAD+ synthesis: structures of *Methanobacterium thermoautotrophicum* NMN adenylyltransferase complexes. J. Biol. Chem. **276**, 7225.
2. Christendat, D., Saridakis, V., Dharamsi, A., Bochkarev, A., Pai, E.F., Arrowsmith, C.H., and Edwards, A.M. (2000) Crystal structure of dTDP-4-keto-6-deoxy-D-hexulose 3, 5-epimerase from *Methanobacterium thermoautotrophicum* complexed with dTDP. J. Biol. Chem. **275**, 24608.
3. Fothergill, M.D. and Fersht, A.R. (1991) Correlations between kinetic and X-ray analyses of engineered enzymes: crystal structures of mutants Cys-Gly-35 and Tyr-Phe-34 of tyrosyl-tRNA synthetase. Biochemistry **30**, 5157.
4. Hendrickson, W.A., Horton, J.R., and LeMaster, D.M. (1990) Selenomethionyl proteins produced for analysis by multiwavelength anomalous diffraction (MAD): a vehicle for direct determination of three-dimensional structure. Embo J. **9**, 1665.
5. Rould, M. (1997) Screening for heavy-atom derivatives and obtaining accurate isomorphous differences. Methods in Enzymology **276**, 112.
6. Mulichak, A.M., Losey, H.C., Walsh, C.T., and Garavito, R.M. (2001) Structure of the UDP-glucosyltransferase GtfB that modifies the heptapeptide aglycone in the biosynthesis of vancomycin group antibiotics. Structure **9**, 547.
7. Stura, E. (1999) Protein crystallization, techniques, strategies, and tips. 1st ed. La Jolla: International University Line.
8. Saridakis, E. and Chayen, N.E. (2000) Improving protein crystal quality by decoupling nucleation and growth in vapor diffusion. Protein Sci. **9**, 755.
9. McPherson, A. (1998) Crystallization of biological macromolecules. 1st ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

10. Chayen, N.E (1999) Protein crystallization, techniques, strategies, and tips. 1st ed. La Jolla: International University Line.
11. Block., H., Kubicek, J., Labahn, J., Roth, U., and Schäfer, F. (2008) Production and comprehensive quality control of human Interleukin-1β: A case study for a process development strategy. Prot. Exp. and Purif. **57**.
12. Jenny, R.J., Mann, K.G., and Lundblad, R.L. (2003) A critical review of the methods for cleavage of fusion proteins with thrombin and factor Xa. Prot. Expr. Purif. **31**, 1.
13. Lewinson, O., Lee, A.T., and Rees, D.C. (2008) The funnel approach to the precrystallization production of membrane proteins. J. Mol. Biol. **377**, 62.
14. Lesley, S.A., and Wilson, I.A. (2005) Protein production and crystallization at the Joint Center for Structural Genomics. JCFG **6**, 71.
15. Caffrey, M. (2008) On the mechanism of membrane protein crystallization in lipidic mesophases. Cryst Growth Des. **8**, 4244.
16. Newman, J., Egan, D., and Walter, T.S., Meged, R., Berry I., Jelloul, M.B., Sussman, J.L., Stuartc, D.I., and Perrakis, A. (2005) Towards rationalization of crystallization screening for small- to medium-sized academic laboratories: the PACT/JCSG+ strategy. Acta Cryst. **D61**, 1426.
17. Newman, J. (2004) Novel buffer systems for macromolecular crystallization Acta Cryst. **D60**, 610.

Ordering Information

Product	Contents	Cat. no.
NeXtal DWBlock Adhesive Foils	100 pierceable/peelable adhesive foils for deep-well blocks	132101
EasyXtal Sealing Tapes (100)	Microplate sealing tapes (100 sheets)	132105
EasyXtal Refill-Hits	50 ml of individual crystallization suite solution	Varies
NeXtal Stock Solutions	50 or 200 ml of crystallization stock solution	Varies
NeXtal CubicPhase Kit	2 x 96-well plates coated with monoolein and 2 deep-well blocks containing 96 x 1.5 ml solutions for crystallization of membrane proteins	130807
NeXtal CubicPhase μplate (10)*	10 x 96-well plates coated with monoolein for crystallization of membrane proteins	130803
Related products		
QIAgenes Expression Kits— for optimized expression of recombinant human proteins in *E. coli*, insect, or mammalian systems		
QIAgenes Expression Kit *E. coli*	QIAgenes Expression Construct *E. coli*, Positive Control, Penta·His Antibody, 4 Ni-NTA Spin Columns	Varies
QIAgenes Expression Kit Insect/Mammalia	QIAgenes Expression Construct Insect/Mammalia, Positive Control, Penta·His Antibody, Ni-NTA Magnetic Agarose Beads	Varies
Ni-NTA Superflow — for efficient immobilized-metal affinity chromatography (IMAC) using FPLC		
Ni-NTA Superflow (25 ml)*†	25 ml nickel-charged resin (max. pressure: 140 psi)	30510

* Other kit/product sizes and/or formats available; see www.qiagen.com.

† Also available as a prefilled cartridge.

Ordering Information

Product	Contents	Cat. no.
Strep-Tactin Superflow Plus — for batch, gravity-flow, or FPLC purification of _Strep_-tagged proteins		
Strep-Tactin Superflow Plus (10 ml)*†	For purification of _Strep_-tagged proteins: 10 ml _Strep_-Tactin-charged Superflow (max. pressure: 140 psi)	30004
Glutathione Superflow— for batch, gravity-flow, or FPLC purification of GST-tagged proteins		
Glutathione Superflow (10 ml)*†	For purification of GST-tagged proteins: 10 ml Glutathione Superflow	30900
Ni-NTA Membrane Protein Kit — for standardized solubilization and purification of membrane proteins		
Ni-NTA Membrane Protein Kit*	For 5 detergent screenings and 5 affinity purifications: 7 detergents, buffers, Ni-NTA Superflow, Penta·His Antibody, disposable columns	30610
EasyXpress Protein Synthesis Mega Kit — for large-scale in vitro synthesis of protein for structural studies		
EasyXpress Protein Synthesis Mega Kit	For 2 x 5 ml reactions: _E. coli_ extract, reaction buffers, amino acid mix w/o methionine, methionine, RNase-free water, gel-filtration columns, and reaction flasks	32516

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

* Other kit/product sizes and/or formats available; see www.qiagen.com.

† Also available as a prefilled cartridge.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the EasyXtal and NeXtal products to the following terms:

1. EasyXtal and NeXtal products may be used solely in accordance with the *EasyXtal and NeXtal Protein Crystallization Handbook* and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of these products with any components not included within these products except as described in the *EasyXtal and NeXtal Protein Crystallization Handbook* and additional protocols available at www.qiagen.com.
2. Other than expressly stated licenses, QIAGEN makes no warranty that these products and/or their use(s) do not infringe the rights of third-parties.
3. These products and their components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of these products agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to these products and/or its components.

For updated license terms, see www.qiagen.com.

www.qiagen.com

Australia ■ Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 021-3865-3865 ■ Fax 021-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 02-33430-420 ■ Fax 02-33430-426 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 1544 7145 ■ Fax 1544 7146 ■ Technical 1544 7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-639

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 65-67775366 ■ Fax 65-67785177 ■ Technical 65-67775366

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

1060049 01/2010

Sample & Assay Technologies