

How a new chemical compatibility test facilitates protein crystallization



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Introduction

Important factors in crystallization screening are:

- The type and concentration of precipitant
- Protein concentration
- pH

A traditional approach in initial crystallization trials is to use one protein concentration for all precipitant types — salts, polymers, and organics — over all pH values. However, the fact that protein solubility usually varies according to precipitant type means that such an approach wastes time and precious protein.

There are three methods available to determine an optimal protein concentration:

Based on past experience

- Amount of protein required dependent on success of screen, can be large
- Quick and easy to set up, highly flexible
- May require multiple rounds

Based on physical parameters of protein in solution (e.g., light scattering)

- Requires expensive equipment
- Provides little information on optimal crystallization conditions

Using the EasyXtal Pre-Screen Assay

- Quick and easy to set up
- Results are immediately applicable to initial screening strategy (i.e., optimal precipitant and protein concentration)

The EasyXtal Pre-Screen Assay saves time and material and increases the probability of nucleation and crystallization events by offering quick and easy determination of the optimal protein concentration for different precipitant types at different pH values.

Results

- With one exception (thaumatin), similar precipitation rates were obtained for all three classes of precipitant when using the pre-screen assay derived protein concentrations.
- Using an arbitrarily chosen protein concentration, only one protein (ADH) displayed similar precipitation rates over the three precipitant classes.
- In the case of thaumatin, the low precipitation rates obtained in organic and polymer precipitants imply that exploring screens based solely on salts offer the best chance of obtaining crystals.

Conclusions

- The EasyXtal Pre-Screen Assay is a simple, fast, and effective tool for determining optimal protein concentrations for initial screening, and provides useful hints for further optimization.
- Combining the pre-screen assay with a pro-active screening strategy greatly increases the chances of detecting favorable conditions, compared to conventional approaches.

Convenient and fast setup and easy interpretation

The EasyXtal Pre-Screen Assay is available ready-to-use in a 24-well EasyXtal DG-Tool (Figure 1A) with 24 X-Seal DropGuard crystallization supports (Figure 1B). Precipitant conditions are arranged in pairs on the plate, at a high and low concentration or pH (see Table). For each well, samples containing different concentrations of protein (e.g., 30, 15, and 7.5mg/ml) are pipetted into separate cavities of a support, mixed with an equivalent volume of precipitant solution, and screwed into place above the reservoir. After a 30–60 minute incubation, each protein drop is inspected for the presence of precipitate. The optimal protein concentration for each precipitant is that which gives a precipitate at the high concentration and a clear drop at the low concentration.



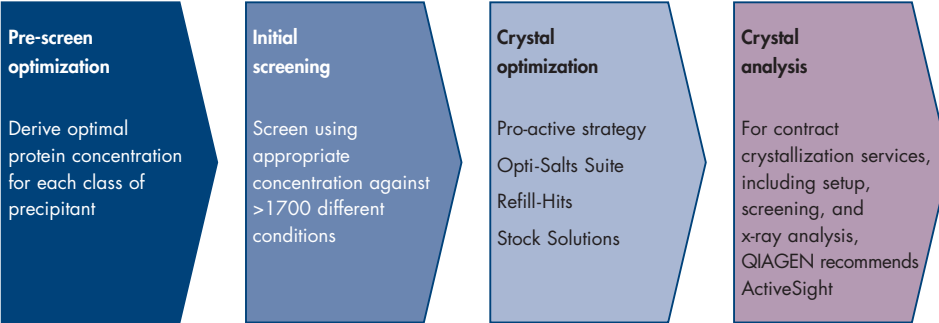
Figure 1 A EasyXtal Pre-Screen Assay 24-well plate and B DropGuard Crystallization Support.

Protein conc. (mg/ml)	Well B1, 3.2 M AmSO ₄	Well B2, 1.1 M AmSO ₄	Protein conc.
7.5 mg/ml			Too low
15 mg/ml			Optimal
30 mg/ml			Too high

	Salts		Organics		Polymers	
	1	2	3	4	5	6
A	4.3 M NaCl	1.5 M NaCl	30% glycerol 2M AmSO ₄	15% glycerol 2M AmSO ₄	30% PEG 400	12% PEG 400
B	3.2 M AmSO ₄	1.1 M AmSO ₄	40% 2-propanol	15% 2-propanol	30% PEG 4000	12% PEG 4000
C	3.0 M Na/K phosphate	1.0 M Na/K phosphate	65% MPD	23% MPD	30% PEG 8000	12% PEG 8000
D	2 M AmSO ₄ , 0.1 M acetate, pH 4.6	2 M AmSO ₄ , 0.1 M Tris, pH 8.5	50% MPD, 0.1 M acetate, pH 4.6	50% MPD, 0.1 M Tris, pH 8.5	20% PEG 4000, 0.1 M acetate, pH 4.6	20% PEG 4000, 0.1 M Tris, pH 8.5

An integrated and structured approach to protein crystallization

- The EasyXtal Pre-Screen Assay is just one part of QIAGEN integrated solutions for protein structural analysis.



Effect of protein concentration optimization on precipitation rates

- Six commercially available proteins were used as test candidates for the EasyXtal Pre-Screen Assay.
- Using the results of the Pre-Screen Assay, protein concentrations that should deliver uniform precipitation rates in each class of precipitant (organics, salts, and polymers) were chosen and used in an initial screen (EasyXtal Classics Suite) containing 96 different conditions.
- The precipitation rates obtained for each protein in each precipitant were compared with those obtained for a single, arbitrarily chosen protein concentration using the same screen.

	PSA-derived, precipitant-specific protein concentrations, Classics Suite			Single, arbitrarily chosen protein concentration, Classics Suite		
Protein	Organics	Salts	Polymers	Organics	Salts	Polymers
ADH	74%	71%	74%	74%	71%	74%
Lipase	61%	58%	56%	61%	3%	6%
Lysozyme	48%	55%	59%	3%	29%	26%
Catalase	42%	45%	32%	10%	35%	9%
Thaumatin	10%	81%	15%	0%	19%	3%
Trypsin	52%	42%	62%	19%	32%	56%

■ <25% or >85% ■ 25–40% or 61–85% ■ 41–60%

References and further reading

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