Analytic Crystallizer Kit

Introduction

The Analytic Crystallizer Kit (Product No. AL-CRYST) comprises a two-step method for optimal crystallization screening of detergent-solubilized integral membrane proteins (IMPs) in a manner independent of detergent identity. The kit includes a solubility prescreen (Optimizer) that is used to determine the appropriate protein concentration for use with the crystallization screen (Crystallizer). The use of Optimizer allows the experimenter to reduce the amount of crystallization conditions to be screened in the first screening pass and to increase the likelihood of crystallization success. The result of coupling the Optimizer and Crystallizer Solutions Sets is the assurance that the majority of the crystallization screen solutions will result in supersaturation of the protein without excessive non-crystalline precipitation.

The conditions for the Crystallizer Kit have been formulated to avoid redundancy and maximize coverage of crystallization space. Precipitants and salts were mined from the conditions most frequently used in successful membrane protein crystallization experiments, as available in the Membrane Protein Data Bank. Also sampled are six buffers ranging from pH 4.5 to 9.5 and 18 distinct salts.

The formulations provided here have been further tuned to render the screen detergent-independent, meaning that the precipitant and salt concentrations have been selected to minimize phase separation that typically occurs for solutions containing high concentrations of detergents that are typically used to maintain the solubility of membrane proteins.

Analytic
crystallizer

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Protocol

Optimizer Protocol for IMP Concentration

1. For all spectrophotometric steps in this protocol, we recommend the use of a NanoDrop™ spectrophotometer, or equivalent.
2. Prior to measurements, blank the spectrophotometer with the current protein buffer, including detergent.
3. Concentrate the sample using a centrifugal concentrator of appropriate molecular weight cut-off, monitoring the protein concentration to achieve 50 µM, which is a concentration at which most IMPs will be soluble and stable.

Technical Tip: *In this step, it is important to agitate the solution close to the concentrator membrane to ensure that the sample is homogenous.*

4. Once the 50 µM concentration is reached, transfer 400 µl of the protein sample to a 0.5 ml concentrator of appropriate molecular weight cut-off.
5. Concentrate to reduce the total sample volume to 200 µl and measure the absorbance at both 280 nm and 320 nm. Make note if the absorbance at 320 nm has increased over that of the original 400 µl sample volume, as this parameter is a good indicator of undesirable protein aggregation.
6. If the absorbance at 320 nm continues to remain low, further reduce the volume of the sample to 100 µl and again measure absorbance at 280 nm and 320 nm.
7. If the absorbance reading at 320 nm remains low, perform an additional concentrate step to reduce the protein volume to 50 µl. Again measure the absorbance at 280 nm and 320 nm. If the reading at 320 nm remains unchanged, take sufficient amount of the sample for 24 experiments in the solubility prescreen, Optimizer. For 100 nl + 100 nl, you would need 3 µl of sample at this stage. For larger drops or manual set-up, 7 µl of retained sample is recommended.
8. Concentrate the remaining volume to 25 µl and, again, measure the absorbance at 280 nm and 320 nm. If the reading at 320 nm shows an increase at this stage, then this is the maximum desired concentration. Use 7 µl of this sample for use in the Optimizer step.

Technical Tip: *For the Optimizer step, it is ideal to have two concentrations to take full advantage of the Optimizer screen, one protein with a slightly increased reading at 320 nm and one concentration at 50% of the first.*

Technical Tip: *The void volume for the 0.5 ml concentrators is usually 20 µl, which makes 25 µl a good stopping point to ensure there is sufficient sample for screening.*

9. For each aliquot of the sample, set the Optimizer screen using equal volumes of protein and the Optimizer Solution.

10. Inspect each Optimizer result immediately post-set-up and after 10 minutes of incubation. **The optimal concentration at which to proceed to the Crystallizer screen will show precipitation in approximately 50% of conditions from Optimizer.**

Technical Tip: *If you observe more than 50% of solutions resulting in protein precipitant, the protein concentration is likely too high to yield usable crystal hits in the Crystallizer screen when using vapor diffusion. You will need to reduce the protein concentration prior to setting Crystallizer in vapor diffusion. Alternatively, you may wish to consider the use of the Crystal Former (Product No. CF-HT2) from Microlytic for screening samples of higher protein concentration as the gradients established in this microfluidic device allow for a more complete overview of crystallization space and protein behavior.*

Trend to monitor: A protein sample that exhibits precipitated drops in conditions containing high PEG concentrations but yields clear drops for low PEG concentrations has been optimally prepared for subsequent crystallization screening.

Contents—Optimizer Formulations

No.	Well	Alt. Well	Salt	Buffer	Precipitant
1	A1	A7			50% (v/v) PEG 400
2	B1	B7			25% (v/v) PEG 400
3	C1	C7			40% (v/v) PEG 550 MME
4	D1	D7			20% (v/v) PEG 550 MME
5	E1	E7	100 mM Lithium Sulfate		35% (w/v) PEG 2000 MME
6	F1	F7	100 mM Lithium Sulfate		17% (w/v) PEG 2000 MME
7	G1	G7			36% (w/v) PEG 1000
8	H1	H7			18% (w/v) PEG 1000
9	A2	A8			35% (w/v) PEG 2000 MME
10	B2	B8			17% (w/v) PEG 2000 MME
11	C2	C8	100 mM Potassium Thiocyanate		35% (w/v) PEG 2000 MME
12	D2	D8	100 mM Potassium Thiocyanate		17% (w/v) PEG 2000 MME
13	E2	E8			25% (w/v) PEG 4000
14	F2	F8			12% (w/v) PEG 4000
15	G2	G8			22% (w/v) PEG 5000 MME
16	H2	H8			11% (w/v) PEG 5000 MME
17	A3	A9	100 mM Magnesium Acetate		35% (w/v) PEG 2000 MME
18	B3	B9	100 mM Magnesium Acetate		17% (w/v) PEG 2000 MME
19	C3	C9		100 mM Sodium Citrate pH 4.5	30% (w/v) PEG 1000
20	D3	D9		100 mM Tris:HCl pH 8.5	30% (w/v) PEG 1000
21	E3	E9		100 mM Sodium Citrate pH 4.5	25% (w/v) PEG 2000 MME
22	F3	F9		100 mM Tris:HCl pH 8.5	25% (w/v) PEG 2000 MME
23	G3	G9	100 mM Ammonium Nitrate		35% (w/v) PEG 2000 MME
24	H3	H9	100 mM Ammonium Nitrate		17% (w/v) PEG 2000 MME

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