



## Three step procedure for evaluating the Crystal Former

In order to evaluate the Crystal Former and compare it to your existing workflow, we suggest that you design a set of experiments for proteins that are:

- (1) Known to produce crystals under well defined conditions in the Crystal Former (Table 1)
- (2) Previously crystallized using your current workflow
- (3) Not crystallized using your current workflow

### (1) Model proteins known to produce crystals in Crystal Former

To familiarize yourself with the features and format of the Crystal Former we suggest that you use one Crystal Former to grow crystals of a suitable model protein (e.g. Lysozyme, Xylanase or Thaumatin). This procedure will allow you to test your loading and sealing technique and to hone your crystal harvesting skills using a non precious protein sample.

Examples of suitable model proteins and their crystallization and cryo conditions are listed in Table 1. Simply load 0.5  $\mu$ L of protein solution and 0.5  $\mu$ L of precipitant solution as described in the user manual. To cryo protect crystals, add 5  $\mu$ L of suggested cryo solution (Table 1) to the opened channel (see user manual for details).

**Table 1: Well characterized crystallization conditions for the Crystal Former**

Test protein	Hen egg white Lysozyme	Xylanase XYNII	Thaumatin
Supplier	Sigma (A7641)	Hampton Research (HR7-104)	Sigma (T7638)
Protein buffer	50 mg/mL in de-ionized water	Dilute to 18 mg/mL in de-ionized water	50 mg/mL in de-ionized water
Precipitant	20 % w/v Sodium chloride in 0.1 M Sodium acetate pH 5.5	0.2 M trimethylamine N-oxide, 0.1 M TRIS pH 8.5, 20 % w/v PEG 2000mme (JCSG plus #76)	0.8 M Potassium sodium tartrate, 0.1 M HEPES pH 7.5 (Crystal Screen 1 #29)
Cryo solution	25% glycerol, 10 % w/v Sodium chloride in 0.05 M Sodium acetate pH 5.5	25% glycerol, 0.1 M trimethylamine N-oxide, 0.05 M TRIS pH 8.5, 10 % w/v PEG 2000mme	25% glycerol, 0.8 M Potassium sodium tartrate, 0.1 M HEPES pH 7.5
Incubation time	1-7 days	3-7 days	3-7 days



## (2) Proteins crystallized using current workflow

Before integrating a new tool in your workflow you need to make sure that you are able to reproduce the successes of your current workflow. Consequently we advise you to use the Crystal Former to re-screen a set of proteins, for which you were able to get crystal hits in your initial screening using your existing workflow.

It is important for this part of the evaluation that you do not simply retest in the Crystal Former the conditions known to give crystals using vapor diffusion. Rather you should re-screen the protein either using your standard sparse matrix screen that was used when screening using your current workflow or using a screen optimized for the Crystal Former such as the SmartScreen or PurePEG. The rationale for this approach is that the diffusive mixing kinetics of the Crystal Former are fundamentally different from vapor diffusion or batch based methods, thus it is not guaranteed that all crystallization conditions giving crystals using batch or vapor diffusion will give crystals using the Crystal Former. In fact, tests have shown that the direct translation of crystallization conditions from vapor diffusion give crystals using the Crystal Former for 60 % of the conditions. However, the direct translation of crystallization hits from the Crystal Former to vapor diffusion gives crystals only for 10 % of the conditions, highlighting the improved crystallization hit rate of the Crystal Former.

We recommend that you select one or two proteins, that have previously been crystallized using your existing workflow, and set up de novo crystallization trials using the Crystal Former and SmartScreen or PurePEGs. From this evaluation you can determine whether you can get equivalent results using the Crystal Former as using your existing workflow.

## (3) Proteins not crystallized using current workflow

The true power of the Crystal Former is the improved crystallization hit rate, which may allow you to get crystals from proteins that you have previously not been able to crystallize. In your evaluation of the Crystal Former we encourage that you select several proteins that are biophysically well behaved, but difficult to crystallize using your current workflow. For these proteins we recommend setting up crystallization trials using the Crystal Former and one or more of the sparse matrix screens that have been optimized specifically for the Crystal Former (e.g. SmartScreen and/or PurePEGs).

From this evaluation you will be able to test whether the unique properties of the Crystal Former can facilitate crystallization of even these difficult to crystallize proteins.

**NOTE ON PROTEIN CONCENTRATION:** Protein concentration in the loading buffer, usually water or dilute buffer, should be approximately 60% – 80% of saturation; at least 10 – 30 mg/mL.