

MOLECULAR DIMENSIONS MONOGRAPH SERIES[®]

Tried your usual crystal growth screens without success?

Here is a quick method for a systematic alternative to screening with sparse matrices that will:

- Identify the limit of nucleation/ precipitation.
- Which crystallization agents to use?
- Which concentration?
- Which pH?

You can do all of this with The Solubility Tool Kit[™]. Ideally it is worth testing crystallization at three pH values, where the protein bears a positive, negative and zero net charge respectively. In The Solubility Tool Kit pH 4.5 and pH 9.0 have been chosen since most proteins will carry a net positive and net negative charge respectively at these values of pH. Buffers are provided for setting up crystallization trials at pH = pI.

The aim of The Solubility Tool Kit is to determine the crystallization potential of protein salts. The search is based on the net charge of the protein and the chemical nature of the crystallizing agent (Hofmeister series). It is more efficient than screening one particular condition of a phase diagram since screening is done with ranges of salt concentrations.

The method:

Set up vapour diffusion drops of the least concentrated salt conditions. Wait for a couple of days, then transfer the coverslip onto the reservoir of next concentration. This way, both protein and crystallising agent concentrate. This method is especially useful when working with low protein concentration (less than 5 mg/ml). This should be done with the six salts of each pH set (pH 4.5 and 9.0).

If sample is limited just one pH condition can be used, or just 2 conditions per pH. For example: sodium chloride and ammonium sulphate at pH 4.5, ammonium sulphate and sodium acetate at pH 9.0.

For the test at pI, because vapour diffusion is very slow with PEG's a quick trial can be done using the batch method. Alternatively, dispense the protein onto the coverslip/microbridge add the precipitant (for this method first choose the second highest concentration in the kit). Do not mix, but go immediately to the microscope. As the precipitant and the protein mix together the protein will experience locally the final precipitant concentration. You should see some precipitation within 5 minutes. If you see too much precipitate set up a lower precipitant concentration and repeat. If after 10 minutes there is absolutely no precipitate consider concentrating your protein more. Otherwise it is advised to set up the trial with dialysis buttons. Set up six drops of the least concentrated salt conditions and move the buttons every 2-3 days to a higher precipitant concentration.

The first aim is to detect the limit of nucleation/precipitation for one or more crystallizing agent(s).

If the range is found, i.e. clear drops followed by precipitates along a salt gradient, prepare a new tray with the salt concentrations ranging from the last concentration where the drop remained clear to the first concentration where precipitation was observed. To do this, prepare a series of reservoirs using the tubes containing the two solutions from both sides of the limit, and prepare a new gradient. Wait one week before drawing any conclusions.

To conserve protein, transfer the coverslips with clear drops onto reservoirs of higher concentration, and those having precipitates to lower concentrations.

Once some solid phase (spherulites, microcrystals, etc.) appears, the conditions must be refined in order to grow large single crystals as done classically by other methods.

Vaney M.C., Broutin I., Retailleau P., Douangamath A., Lafont S., Hamiaux T., Prange T., Ducruix A. & Riès-Kautt M. Structural effects of monovalent anions on polymorphic lysozyme crystals. *Acta Cryst.* (2001) **D57**, 929-940.