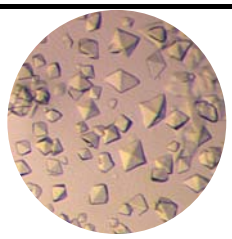
Molecular Dimensions



MicroSeed Beads MD2-14

For the production of microseeds for optimization experiments.

MD2-14 is presented as 24 PTFE MicroSeed Beads contained in individual 1.5 mL Microfuge tubes.

Features of MicroSeed Beads:

- Improve the size of crystals or control the number of crystals.
- Get more consistent results.
- Speed up results if spontaneous nucleation is slow.
- Avoid cross-contamination unlike glass beads.
- Obtain a wider range of crystal forms (polymorphs) by seeding into totally different precipitants.

MicroSeed Beads:

MicroSeed Beads are used to generate microseeds (sub-microscopic crystals that have been 'crushed-up' into fragments).

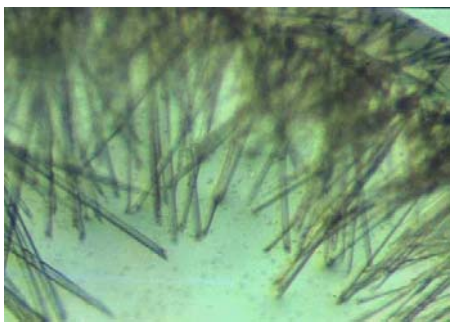
Crystals are crushed using the PTFE balls and suspended into serial dilutions of mother liquor. The actual seeds are invisible with an optical microscope.

What is seeding?

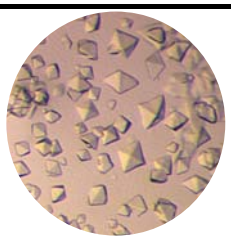
Seeding is the use of an existing nucleus (usually a small crystal or crystal fragment), introduced into a new drop, where it acts as a growth site.

Seeding is used to separate nucleation from growth, manipulating crystal growth in the metastable zone and bypassing the need for spontaneous nucleation. It is easier to add onto an already existing nucleus than create one *de novo*. This separation is achieved by transferring a seed (sub-microscopic crystal) from one condition, where the level of supersaturation is high, to a similar condition at a lower level of supersaturation.

Figure 1 (overleaf) shows a representation of a crystallization experiment showing results with and without a macroseed.



The effect of Microseeding – IspD protein before microseeding growing in clusters on the edge of the drop (left) and after Microseeding the crystals grow separately (right).
(Images courtesy of Terese Bergfors).



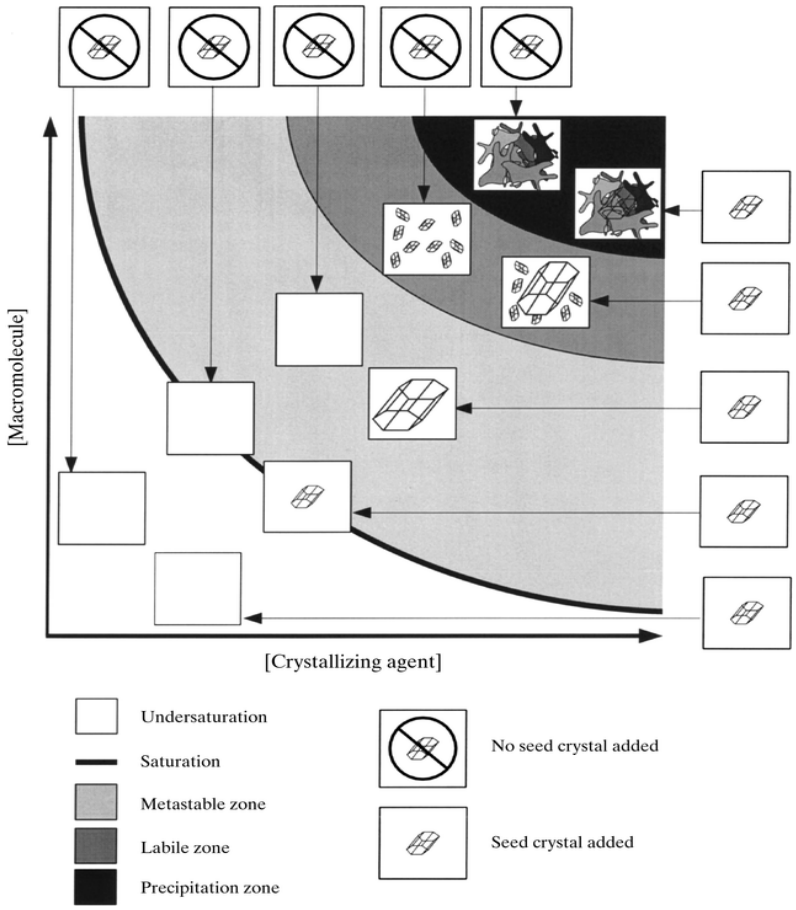


Figure 1: Seeding and the phase diagram. From Luft and DeTitta, Acta Cryst. (1999) D55, 988-993.

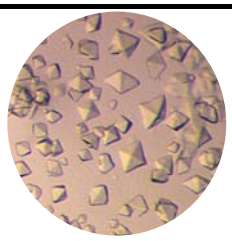
MicroSeeding and the Phase Diagram:

Metastable Zone: Critical nucleation has not yet been reached. Spontaneous nucleation does not occur unless it is introduced by vibrations (i.e. crystal plate being knocked, incubator vibrations etc.) or introduction of a particle that will promote heterogeneous nucleation (i.e. a seed or nucleant). This region is the 'ideal' region to be in for microseeding.

Nucleation Zone (labile zone): Crystal appears spontaneously in this zone. It is important to control the nucleation. If supersaturation is increased the number of crystals increases and they appear earlier. Microseeding aims to avoid the nucleation zone. If seed crystals are added to this zone- it can 'shock' the solution and cause excessive nucleation.

Precipitation Zone:
Precipitation occurs at very high supersaturation. Insoluble protein separates from the solution into the amorphous state.

Molecular Dimensions



What you need before you begin:

1. MicroSeed bead and microfuge tube.
2. Protein crystal sample.
3. 150 µL Stabilizing solution (this is a mixture of a ratio of 1:5:4 concentrated protein: original crystallization solution: water. It is advisable to test the stability of your crystal in this stabilization solution prior to seeding).
4. Something to aspirate your crystal with and place in the microfuge tube e.g. Glass capillary/pipette bulb/0.1-2 µL pipette and tips or paper wick.
5. At least six empty microfuge tubes for serial dilutions or a PCR tube row/plate.

Making your Seed Stock:

1. Take 50 µL of the stabilizing solution and pipette into a MicroSeed bead microfuge tube.
2. Transfer a single crystal or stack of crystals to the MicroSeed Bead tube by aspirating a drop that contains the crystal/s.
3. Make your seed stock by vortexing the MicroSeed Bead tube for 90 secs to pulversise the crystal. IMPORTANT: Set the vortexer at a mid-point setting on the vortexer. A too low vortex speed will mean the crystal will not be crushed.
4. Prepare a serial dilution of the seed stock into either six microfuge tubes or PCR tubes/plate. The standard dilution series suggested is 1 in 4-fold dilution (5 µL seeds + 15 µL stabilizing solution for each dilution). See Figure 2.

Setting-up crystallization drops:

Microseeding experiments can be carried out in either sitting-drop or hanging-drop experiments and done by hand or robotics.

The following are guidelines for a typical microseeding experiment and can be altered as necessary.

By Robot:

Take a 96-well crystallization plate, e.g. 96-well MRC plate and pipette out 100 µL of a 70% solution of the original crystallization condition into the reservoir wells.

Dispense the diluted seeds together with protein samples into the sample wells - a volume of 100 nL + 100 nL is suggested.

It is a good idea to try all dilutions of the seed stocks- if protein sample permits.

By Hand:

Use 1 mL of a 70% solution of the original crystallization condition into the reservoir wells. Dispense 1 µL + 1 µL of protein sample + diluted seed solutions into the sample wells or onto hanging-drop coverslips.

Notes:

If you have detergents or other additives that may foam then it is advisable not to use a vortex to pulverise your crystals. An ultrasonic cleaner is the best method to use.

A new microfuge tube and PTFE MicroSeed bead should be used for each new experiment to avoid cross-contamination.

Seed stocks and their dilutions maybe frozen for later use.

Additives can be added to the frozen seeds at the appropriate dilution to optimize crystallization with additives.

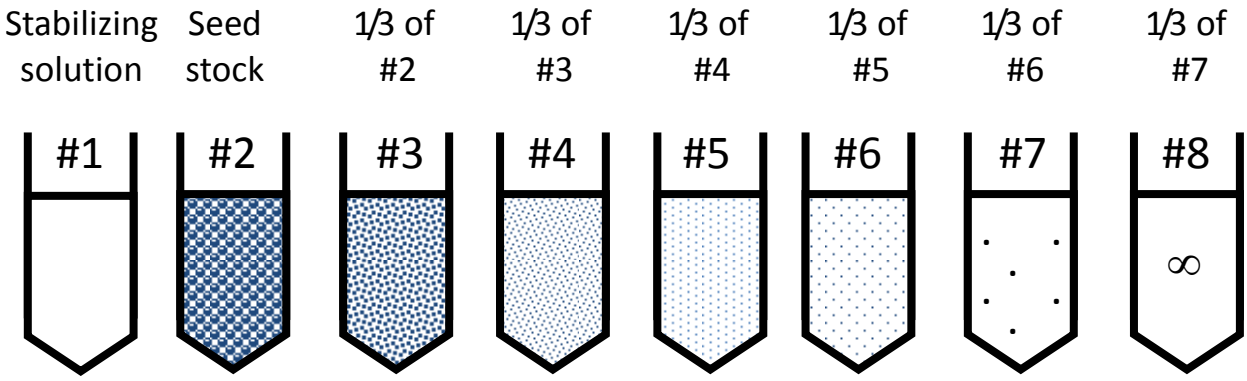
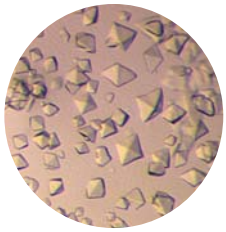


Figure 2. Serial dilution of seed stock (#2) using a 1 in 4 dilution showing serial dilutions until infinite dilution (#8).



References:
Luft and DeTitta, Acta Cryst. (1999) D55, 988-993.

Further Reading:
Stura E.A., Wilson I.A. Analytical and Production Seeding Techniques. (1990) Methods 1:38 - 49.
D'Arcy A., Villard F., and Marsh M. *Acta Cryst.* (2007). D63, 550-554.
Walter T.S., Mancini E.J., Kadlec J., Graham S. C., Assenberg R., Ren J., Sainsbury S., Owens R.J., Stuart D. I., Grimes J.M., and Harlos K., *Acta Cryst.* (2008). F64, 14-18.

Ordering details:

Catalogue Description	Catalogue Code
MicroSeed Beads	MD2-14

A last word from Dr.Enrico Stura...

This thing all things devours:
Birds, beasts, trees, flowers;
Gnaws iron, bites steel;
Grinds hard stones to meal;
Slays king, ruins town
And beats high mountains down.

Answer: TIME
(From J.R.R.Tolkein, The Hobbit)

In all crystallization processes the most important factor is TIME: in equilibration processes, when scaling up and most important in seeding. The environment may be thought to be the same, but the rate at which it changes is not. Crystal growth takes TIME, transporting macromolecules to the crystal's surface takes TIME. The rate at which drop-volume changes will be affected by the drop size and this will affect supersaturation in a time-dependent manner.
Those who understand how to control TIME in the crystallization drop, will "run with the Red Queen" to remain in the same place. In seeding, when scaling up, one may need to change the drop-reservoir salt ratio to achieve the same environment that exists in smaller drops.