Microbatch Crystallization

HAMPTON RESEARCH
Solutions for Crystal Growth

Method

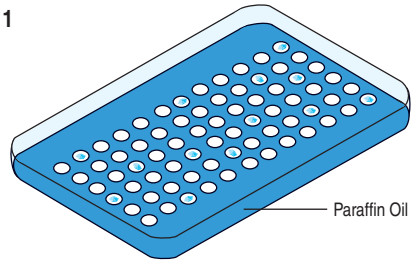
Crystallization under oil where a small drop of sample combined with the precipitant of choice is pipetted a layer of oil. This is known as Microbatch Crystallization.

Oils can also be used as a barrier between the reservoir and the drop in traditional Hanging or Sitting Drop crystallization experiments. This is known as Vapor Diffusion Rate Control.

Description of Microbatch

The crystallization of proteins under a thin layer of Paraffin Oil was originally described by Chayen et al (Appl. Cryst. 23 (1990) 297). In this technique a small drop of sample combined with the precipitant of choice is pipetted under a small layer of Paraffin Oil (Figure 1) HR3-411. The oil generally used is a mineral oil of branched paraffins in the $C_{20}+$ range and allows for little to no diffusion of water through the oil. Essentially a batch, or microbatch experiment, all of the reagents involved in the crystallization are present at a specific concentration and no significant concentration of the protein nor the reagents can occur in the drop.

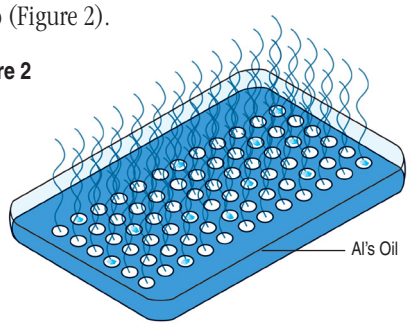
Figure 1



Description of Microbatch - *modified*

D'Arcy et al (A novel approach to crystallizing proteins under oil, Journal of Crystal Growth 168 (1996) 175-180) modified the microbatch under oil technique by using silicone fluids which are polymeric compounds composed of repeating dimethylsiloxane units $-(Si(CH_3)_2-O-)_n-$. Using a mixture of 1:1 Silicon Oil (HR3-415) and Paraffin Oil (HR3-411), also known as Al's Oil (HR3-413), one can perform a microbatch experiment under oil and have diffusion of water from the drop through the oil, hence a microbatch experiment that does allow for concentration of the sample and the reagents in the drop (Figure 2).

Figure 2



Performing Microbatch / Microbatch - *modified*

Pipet 6 milliliters of 100% Paraffin Oil (HR3-411) or 6 milliliters of 1:1 Paraffin/Silicon Oil (Al's Oil HR3-413) into a 72 well Microbatch plate (HR3-120) as shown in figure 1 or 2. Note: one can also utilize other ratios of Paraffin Oil (HR3-411) and Silicon Oil (HR3-415) to vary the rate of diffusion from the drop (higher % of Silicon Oil = more rapid diffusion and evaporation). Use the Combo Pack to create custom oil ratios.

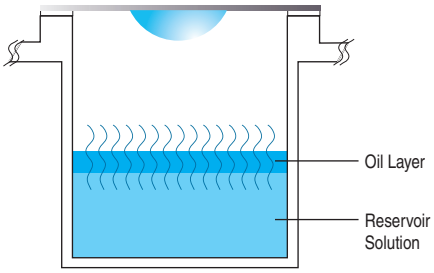
Pipet the sample into the appropriate cone shaped depression in the Microbatch plate followed by addition of the precipitant. Typical drop ratios and sizes are 1:1 and 2 microliters. Drops up to 10 microliters can be achieved under oil using the Microbatch plate. Place plate cover over Microbatch plate to prevent dust and debris from entering experiment.

Description of Vapor Diffusion Rate Control

Chayen (A novel technique to control the rate of vapour diffusion, giving larger protein crystals J. Appl. Cryst 30 (1997) 198-202) has described a technique where oils can be used to vary the rate of vapor diffusion. Using mixtures of Paraffin and Silicon Oil, Chayen reported fewer, larger crystals in the drop.

Using a standard hanging or sitting drop vapor diffusion set up, 200 microliters of oil is applied over the reservoir solution (after the drop is mixed with reservoir solution thus preventing oil from entering the drop) (Figure 3). The oil acts as a barrier to vapor diffusion between the reservoir and the drop. Using 100% Paraffin Oil allows limited amount of vapor diffusion that the drop, thus behaves as a batch experiment and eventually drying up due to evaporation through the polystyrene plate. Using 100% Silicon Oil will give results similar to that when no oil is used. When using a mixture of the two oils the rate of vapor diffusion between the drop and the reservoir may be controlled. The rate of vapor diffusion is also a function of thickness of the oil layer over the reservoir. Chayen evaluated oil volumes between 100 and 700 microliters. Oil volumes of 50 to 100 microliters resulted in crystals similar to the control without oil. Oil volumes greater than 100 to 700 microliters has a significant delay in the onset of crystallization, with improved crystal size. Results using hanging drop were more pronounced than sitting drops which may be due to either surface effects or the drop geometry in relation to the reservoir which could influence vapor diffusion kinetics.

Figure 3



Performing Vapor Diffusion Rate Control

Prepare a VDX (HR3-140) or Cryschem Plate (HR3-158) for a sitting or hanging drop vapor diffusion experiment. After the reservoir has been added to the drop, pipet between 200 and 700 microliters of a mixture of Paraffin/Silicon Oil (HR3-413) onto the reservoir (Figure 3). Seal the plate.

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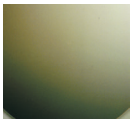
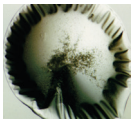
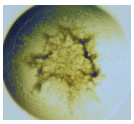
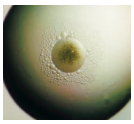
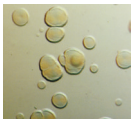
Figure 4
Typical observations in a crystallization experiment

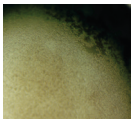

Clear Drop


Skin / Precipitate

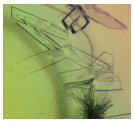

Precipitate


Precipitate / Phase

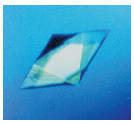

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

Examine The Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week there after. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 4 (on page 2) shows typical examples of what one might observe in a crystallization experiment.

Interpreting The Results

Clear drops indicate that either the relative supersaturation of the sample and reagent is too low or the drop has not yet completed equilibration. If the drop remains clear after 3 to 4 weeks consider repeating the screen condition and doubling the sample concentration.

Drops containing precipitate indicate that either the relative supersaturation of the sample and reagent is too high, the sample has denatured, or the sample is heterogeneous. To reduce the relative supersaturation, dilute the sample twofold and repeat the screen condition. If sample denaturation is suspect, take measures to stabilize the sample (add reducing agent, ligands, glycerol, salt, or other stabilizing agents). If the sample is impure, aggregated, or heterogeneous take measures to pursue homogeneity. It is possible to obtain crystals from precipitate so do not discard nor ignore a drop containing precipitate. If possible, examine drops containing precipitate under polarizing optics to differentiate precipitate from microcrystalline material.

Compare the observations between the 4°C and room temperature incubation to determine the effect of temperature on sample solubility. Different results in the same drops at different temperatures indicate that sample solubility is temperature dependent and that one should include temperature as a variable in subsequent screens and optimization experiments.

Retain and observe plates until the drops are dried out. Crystal growth can occur within 15 minutes or one year.

Related Products

HR3-081	72 Well Untreated, Hydrophobic, Terasaki Style Microbatch Plate, Greiner 654102- 270/case
HR3-121	72 Well Treated, Hydrophilic, Terasaki Style Microbatch Plate, Greiner 654180 - 270/case
HR3-293	Imp@ct plate - 40/case
HR3-295	Imp@ct plate - 10/case
HR3-411	Paraffin Oil - 250 milliliters
HR3-421	Paraffin Oil - 1 liter
HR3-413	Al's Oil (1:1 Paraffin:Silicon) - 250 milliliters
HR3-415	Silicon Oil - 250 milliliters
HR3-423	Silicon Oil - 1 liter
HR3-417	Crystal Oil Combination Pack - one of each oil above

Technical Support

Inquiries regarding Under Oil Crystallization and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 4:30 p.m. USA Pacific Standard Time.

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