Microbatch Crystallization

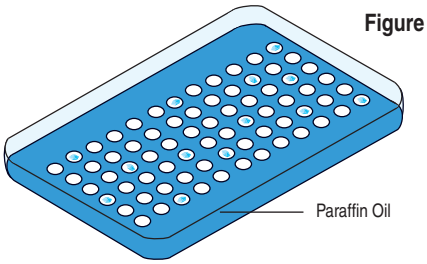
HAMPTON RESEARCH
Solutions for Crystal Growth

Method

Microbatch crystallization is a method where the sample and reagent are combined and sealed in a plate, tube, container, or sealed under a layer of oil.

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.

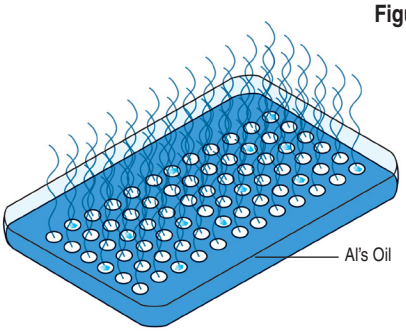
Figure 1



Microbatch Under Oil

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 crystallization reagent 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 2



Modified Microbatch

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).

Using the MRC Under Oil Plate for Microbatch Under Oil & Modified Microbatch

Use a robot or pipette to dispense 20 µl of oil into each of the 96 wells of the MRC Under Oil Plate (HR3-104). Use a robot or pipette to dispense 100 to 200 nl of reagent plus 100 to 200 nl of sample so the drop is completely covered by oil.

Microbatch - Use Paraffin Oil and seal with optically clear tape or film for microbatch with little to no evaporation from the drop (fixed reagent and sample concentration). Access wells and mount crystals by cutting the seal.

Modified Microbatch - Al's Oil or Silicon Oil without a seal for Modified Microbatch controlled evaporation (increased sample and reagent concentration) and view the drops daily for up to a week before the drop completely evaporates.

Using the 72 Well Plate for Microbatch Under Oil & Modified Microbatch

Pipette 6 ml of Paraffin Oil (Microbatch) or 6 ml of Al's Oil or Silicon Oil (Modified Microbatch) into a 72 well Microbatch plate (HR3-081 or HR3-121) as shown in figure 1 or 2. Note: one can also utilize other ratios of Paraffin Oil and Silicon Oil to manipulate the rate of drop evaporation; higher % of Silicon Oil = higher rate of drop evaporation.

Pipette the reagent and sample into the conical flat bottom well of the Microbatch plate. Typical drop ratios and final drop sizes are 1:1 and 1 to 2 µl when set using a manual pipette. Drops up to 10 µl are possible with this plate. Vary drop ratio to evaluate different reagent and sample concentrations. Place plate cover over Microbatch plate to slow drop evaporation and prevent debris from entering experiment.

Microbatch without Oil

Microbatch can also be performed without oil. Batch crystallization experiments used for small molecules typically involved larger volumes than those used for proteins, on the order of tens or hundreds of milliliters, oftentimes in a covered beaker, rather than micro- or nanoliters. Today, some bioprocess protein crystallization experiments employ much larger volumes still, many liters. Neither of these methods utilize oil to cover the protein and reagent. Such experiments are performed in a sealed container, with or without the possibility of evaporation, and can often involve temperature manipulation and control. Microbatch without oil can also be accomplished on a micro- or nanoliter scale in a sealed plate, and is termed Drop Drop crystallization.

Using the Drop Drop Method - Microbatch Without Oil

Use a robot to dispense 400 nl of sample and 400 nl of reagent into each of the 96 wells of the MRC Under Oil Plate (HR3-104). Promptly seal the plate using optically clear film or tape. View drops daily for up to 21 days before the drop completely evaporates. Access wells and mount crystals by cutting the seal. See Image 1 for an example of a Drop Drop Experiment.

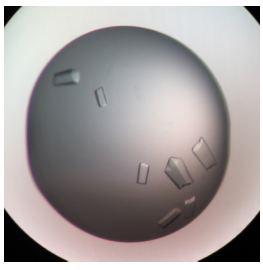


Image 1. Drop Drop experiment with 400 nl protein plus 400 nl reagent and no oil using the MRC Under Oil 96 Well Crystallization Plate.

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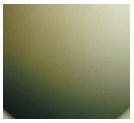
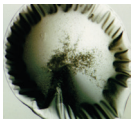
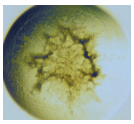
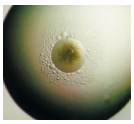
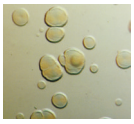
Figure 4
Typical observations in a crystallization experiment

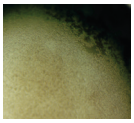

Clear Drop


Skin / Precipitate

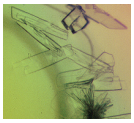

Precipitate


Precipitate / Phase

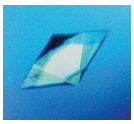

Quasi Crystals


Microcrystals


Needle Cluster


Plates


Rod Cluster


Single Crystal

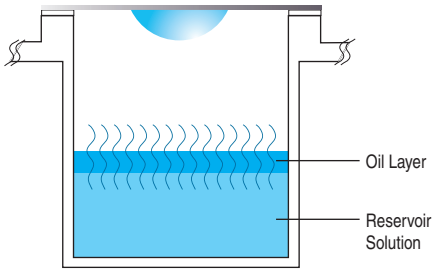
The Drop Drop method can also be used with some 96 well sitting drop vapor diffusion plates, such as the MRC 2 Well Crystallization Plate (HR3-083). Without pipetting reagent into the reagent well, use a robot to dispense 400 nl of sample and 400 nl of reagent into the drop well. Promptly seal the plate using optically clear film or tape. View drops daily for up to 21 days before the drop completely evaporates. Access wells and mount crystals by cutting the seal.

Since the drop dries at a higher rate than a vapor diffusion experiment and there is no reservoir to manage or stop the dehydration of the drop, one must be on the lookout for false positive salt, poymer, or detergent crystals. A disadvantage? Perhaps, but the Drop Drop method allows one to explore a unique path on the solubility curve, as well as use a minuscule amount of crystallization reagent, as well as not leave one with clear drops; eventually, something will happen in the drop, be it a crystal of the sample, of salt, phase separation, or precipitate.

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 between the drop and the reagent well. Using Paraffin Oil, Silicon Oil, or Al's Oil, fewer, larger crystals could be observed in the drop.

Figure 3



Using a standard hanging or sitting drop vapor diffusion set up, oil is pipetted 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. Using 100% Silicon Oil will give results similar to that when no oil is used. When using Al's Oil 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. Oil volumes between 100 and 700 microliters were evaluated. Oil volumes of 50 to 100 microliters resulted in crystals similar to the control without oil. Oil volumes greater than 100 and up 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.

Performing Vapor Diffusion Rate Control

Prepare a VDX hanging drop plate or Cryschem sitting drop plate for a vapor diffusion experiment. After the reagent well has been added to the plate, pipette between 200 and 700 µl of oil onto the reagent in each well. Seal the well or plate.

Plates & Oils for Microbatch

96 well

HR3-102 Swissci MRC Under Oil 96 Well Crystallization Plate 10 plate case

HR3-104 Swissci MRC Under Oil 96 Well Crystallization Plate 40 plate case

• Seal with HR4-521 ClearSeal Film (100 pack), using HR4-413 Film Sealing Paddle (5 pack), or HR3-609 Crystal Clear Sealing Film (100 pack), or HR4-506 Crystal Clear Sealing Tape (3 inch x 55 yard roll, without cutter).

72 well

HR3-081 72 Well Microbatch Plate, Greiner 654102 untreated, hydrophobic - 270 plate case

HR3-121 72 Well Microbatch Plate, Greiner 654180 treated, hydrophilic - 270 plate case

• Supplied with cover, no sealing required.

Oils

HR3-411	Paraffin Oil	100% - 250 ml
HR3-421	Paraffin Oil	100% - 1 L
HR3-415	Silicon Oil	100% - 250 ml
HR3-423	Silicon Oil	100% - 1 L
HR3-413	Al's Oil	(50:50 Paraffin:Silicon) - 250 ml
HR3-417	Combo Oil Pack	(Paraffin, Al's, & Silicon Oil) - 250 ml of each

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