

Crystallization Under Oil

HAMPTON
RESEARCH

Solutions for Crystal Growth

Crystal Growth 101

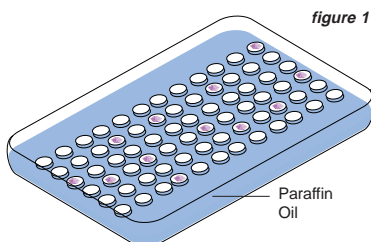
Method

Crystallization under oil is a method where a small drop of sample combined with the crystallization reagent of choice is pipetted under a layer of oil. This is also 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

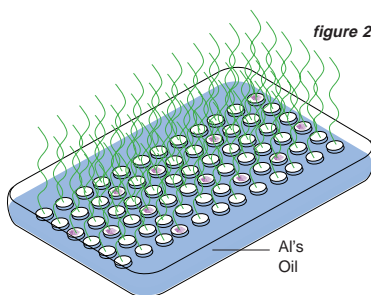
The crystallization of proteins under a thin layer of Paraffin Oil was originally described by Chayen et al. (Appl. Cryst. 23 (1990) 297). In this technique a small drop of sample combined with the reagent of choice is pipetted under a small layer of Paraffin Oil (figure 1) HR3-411. The oil generally used is a mineral oil of branched paraffins in the C₂₀₊ 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.



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).



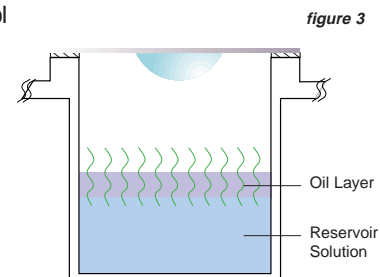
Preforming MicroBatch / MicroBatch - modified

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).

Pipet the sample into the appropriate cone shaped depression in the MicroBatch plate followed by addition of the reagent. Typical drop ratios and final drop sizes are 1:1 and 1 to 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. The MicroBatch method can also be performed in round bottom, clear 96 well plates. Using an 8 or 12 channel pipettor, oil, reagent, and sample can be mixed quickly and efficiently for fast throughput.

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.

Preforming 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.

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 5:00 p.m. USA Pacific Standard Time.

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