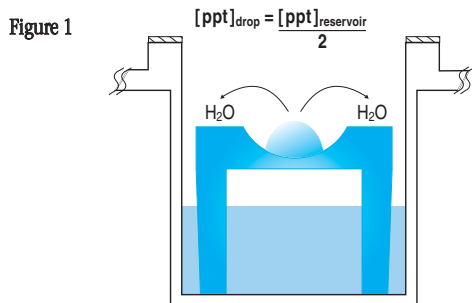


Method

The vapor diffusion technique is the most popular method for the crystallization of macromolecules. The principle of vapor diffusion is straightforward. A drop composed of a mixture of sample and reagent is placed in vapor equilibration with a liquid reservoir of reagent (Figure 1). Typically the drop contains a lower reagent concentration than the reservoir. To achieve equilibrium, water vapor leaves the drop and eventually ends up in the reservoir. As water leaves the drop, the sample undergoes an increase in relative supersaturation. Both the sample and reagent increase in concentration as water leaves the drop for the reservoir. Equilibration is reached when the reagent concentration in the drop is approximately the same as that in the reservoir.



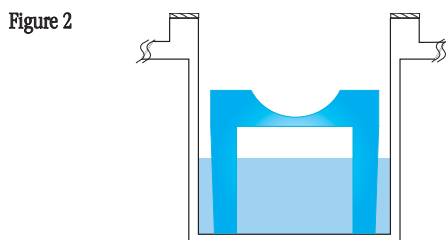
Two simple methods for performing vapor diffusion are sitting drop and hanging drop. The following describes the sitting drop vapor diffusion using the Micro-bridges[®].

Description

The Micro-Bridge is a small bridge (inverted U) manufactured from clear polystyrene which contains a smooth, concave depression in the center of the top region of the bridge. The Micro-Bridge can hold up to 40 microliter drops. The Micro-Bridge is inserted into the reservoirs of VDX or Linbro Plates to perform a sitting drop vapor diffusion experiment. The design of the Micro-Bridge is such that the bridge is quite stable in the reservoir and does not require the Micro-Bridge to be bonded to the plate. The Micro-Bridge can be removed from the plate for crystal manipulation and observation if desired.

Using the Micro-Bridge

1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of a VDX or Linbro plate. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)
2. Place a clean (blow the Micro-Bridge with clean, dry compressed air before use) Micro-Bridge into the bottom of reservoir A1 such that the concave depression in the Micro-Bridge is facing up (Figure 2).



3. Pipet 2 microliters of sample into the Micro-Bridge in reservoir A1. (Note: Recommended total drop volume is 1 to 40 microliters)
4. Pipet 2 microliters of reagent from reservoir A1 into the drop in the Micro-Bridge A1. (Note: Some people prefer to mix the drop while others do not. Proponents of mixing leave the pipet tip in the drop while gently aspirating and dispensing the drop with the pipet. Mixing ensures a homogenous drop and consistency drop to drop. Proponents of not mixing the drop simply pipet the reagent into the sample with no further mixing).
5. Repeat steps 1 through 3 for the remaining 23 reservoirs.
6. Seal the plate with strips of clear sealing tape or plain glass cover slides.

Variations

A. Pipet all 24 reservoirs before placing the Micro-Bridges and pipetting drops. This provides for less evaporation from the drops since the overall time that the drops are exposed to an unsealed environment is less than the above protocol.

B. Pipet all 24 reservoirs before pipetting drop and seal the first two rows (12 reservoirs - A1 thru A6 and B1 thru B6) with one strip of clear sealing tape. This method further reduces drop exposure to evaporation.

C. Seal each reservoir with a plain 22 mm circle or square cover glass and high vacuum grease after pipetting each drop. This method reduces drop exposure beyond variations A and B but removes the convenience of using clear sealing tape. When using this method it is recommended that one apply the high vacuum grease prior to pipetting the reagent unless one is using the Pre-greased VDX Plate. High vacuum grease may be applied by the following method. Fill a 20 cc syringe with high vacuum grease (remove plunger and luer lock seal and squeeze in the grease). Attach a 200 microliter tip (typically yellow) to the luer end of the syringe. Trim the tip of the pipet tip to create an opening which will generate the desired bead size of high vacuum grease. Use all your might and depress the syringe to apply the high vacuum grease. Create a circular bead on the upper edge of the reservoir. Do not complete the circle. Leave a 2 mm opening between the start and finish of the circular bead. Apply the cover slide, press to relieve the air pressure and twist to close the gap.

Micro-Bridge Tips

1. Use Crystal Clear Sealing Tape. Other Brands are optically inferior.
2. To access a drop and/or reservoir of a sealed with tape simply make a circular incision in the tape using the inside of the reservoir as a guide. Use a sharp blade to cut the tape and hold the incised piece of tape with forceps. The opening can be sealed with another strip of tape or a plain 22 mm circle or square cover glass and high vacuum grease.
3. Micro-Bridges can be removed for crystal seeding, mounting, manipulation, and observation.

4. Micro-Bridges are designed as disposable devices and Hampton Research does not recommend washing and re-using Micro-Bridges.

5. Micro-Bridges cannot be siliconized nor autoclaved.

Related Products

HR3-110 Linbro Plate - 50 plate sampler

HR3-112 Linbro Plate - 100 plate case

HR3-140 VDX Plate - 100 plate case

HR3-142 VDX Plate - 24 plate sampler

HR3-170 VDX Plate Greased - 100 plate case

HR3-172 VDX Plate Greased - 24 plate sampler

HR3-310 Micro-Bridges - 100 pack sampler

HR3-312 Micro-Bridges - 400 pack

HR3-340 Micro-Bridges Polypropylene - 100 pack sampler

HR3-342 Micro-Bridges Polypropylene - 400 pack

HR3-211 22 mm Plain Square - 1.0 ounce case

HR3-213 22 mm Plain Square - 10.0 ounce case

HR3-227 22 mm Plain Circle - 1.0 ounce case

HR3-229 22 mm Plain Circle - 10.0 ounce case

HR3-508 Dow Corning® 7 Release Compound Grease

HR3-510 Dow Corning® Vacuum Grease

HR3-511 1.88" Wide Crystal Clear Sealing Tape, with cutter

HR4-506 3" Wide Crystal Clear Sealing Tape

HR4-511 1.88" Wide Crystal Clear Sealing Tape

Technical Support

Inquiries regarding Micro-Bridges 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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