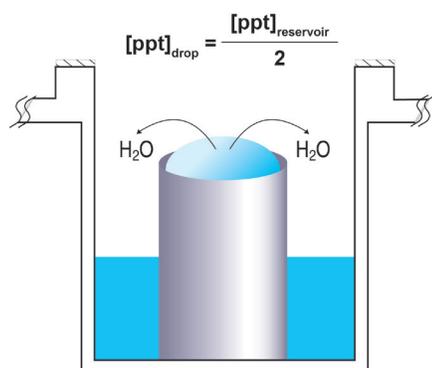


# Sitting Drop Vapor Diffusion Crystallization

The sitting drop vapor diffusion technique is a 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. 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.

Figure 1  
Process of Vapor Diffusion



## Benefits of Sitting Drop Crystallization

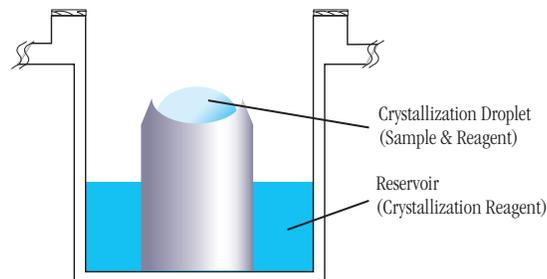
- Can be cost effective.
- Can be time efficient.
- Often easier when using detergents, organics and hydrophobic reagents.
- Drops can be positioned in a stable sitting position.
- Compatible with gels.

## Using the Cryschem™ Plate

The Cryschem Plate is a 24 well plate manufactured from clear polystyrene. Each well contains a post in the center which is elevated above the bottom of the reservoir. The smooth, concave depression in the post can hold up to 40 µl drops and the reservoir can hold up to 1.2 ml of reagent. The Cryschem Plate is sealed with clear sealing tape or film. Rows are labeled A-D and columns are labeled 1-6.

1. Pipet 0.5 ml of crystallization reagent into reservoir A1 of the Cryschem Plate. (Note: Recommended reservoir volume is 0.5 to 1.0 ml)
2. Pipet 2 µl of sample into the post of reservoir A1. (Note: Recommended total drop volume is 0.1 to 40 µl)
3. Pipet 2 µl of reagent from reservoir A1 into the drop in post A1. (Note: Some 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).
4. Repeat steps 1 through 3 for the first two rows of wells so that reservoir and post A1 - B6 are complete.
5. Seal the first two rows with 1.88 inch wide Crystal Clear Sealing Tape.
6. Repeat steps 1 - 5 for reservoir and post C1 - D6.

Figure 2



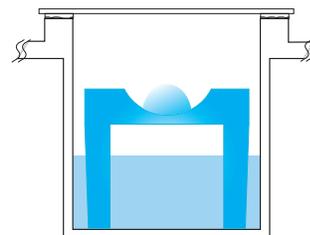
## Cryschem Plate Tips

- Use 1.88 inch wide Crystal Clear Sealing Tape to seal the Cryschem plate two rows (12 reservoirs) at a time.
- Use 3 inch wide Crystal Clear Sealing Tape or Crystal Clear Sealing Film or ClearSeal Film to seal the Cryschem M and Cryschem S plate four rows (all 24 reservoirs) at a time.
- To access a drop and/or reservoir of a Cryschem Plate sealed with tape, make a circular incision in the tape using the X-Acto Gripster Knife and the inside of the reservoir as a guide. Use the X-Acto Gripster Knife to cut the tape and hold the incised piece of tape with forceps. The opening can be sealed with Crystal Clear Sealing Tape or a 22 mm circle or square glass cover slide and vacuum grease.

## Using Micro-Bridges®

The Micro-Bridge is a small bridge (inverted U) manufactured from clear polystyrene or clarified polypropylene which contains a smooth, concave depression in the center of the top region of the bridge (figure 3). The Micro-Bridge can hold up to 40 µl drops. It is inserted into the reservoirs of VDX™ plates to perform a sitting drop vapor diffusion experiment. The design 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.

Figure 3



1. Pipet 1.0 ml of crystallization reagent into reservoir A1 of a VDX Plate with Sealant. (Note: Recommended reservoir volume is 0.5 to 1.0 ml)
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.

# Sitting Drop Vapor Diffusion Crystallization

3. Pipet 2  $\mu$ l of sample into the Micro-Bridge in reservoir A1. (Note: Recommended total drop volume is 0.1 to 40  $\mu$ l)
4. Pipet 2  $\mu$ l of reagent from reservoir A1 into the drop in the Micro-Bridge A1. (Note: Some 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. Using a 22 mm diameter circle cover slide, seal reservoir A1.
6. Repeat steps 1 through 5 for the remaining 23 reservoirs.

## Micro-Bridge Tips

- Micro-Bridges can also be set up in a VDX Plate without sealant and sealed with two strips of 1.88 inch wide Crystal Clear Sealing Tape.
- To access a drop and/or reservoir sealed with tape, make a circular incision in the tape using the X-Acto Gripster Knife and the inside of the reservoir as a guide. Use the X-Acto Gripster Knife to cut the tape and hold the incised piece of tape with forceps. The opening can be sealed with a Crystal Clear Sealing Tape or a 22 mm circle or square glass cover slide and vacuum grease.
- Micro-Bridges can be removed for crystal seeding, mounting, manipulation, and observation.
- Micro-Bridges are designed as disposable devices. It is not recommended to wash and re-use Micro-Bridges.
- Micro-Bridges cannot be siliconized or autoclaved.

## Sandwich Box™

The Sandwich Box consists of a square polystyrene box, a plastic support, and a siliconized 9 well glass plate. The Sandwich Box is used when a common dehydrant system is desired as well as very large drops. Enormous drops can be pipetted into the siliconized glass wells. The siliconized glass plates offer excellent optics and can be removed from the plastic box to inspect the drop for birefringence without optical interference from plastic. Sandwich Boxes offer unique vapor equilibration kinetics and are very easy to access for crystal seeding, manipulation, and mounting. The plates are often used for heavy atom screening and derivatization and are useful for long-term crystal storage when each well is sealed with a glass slide and vacuum grease.

Open the Sandwich Box and place a plastic support, bottom side facing up into the box. Apply a bead of vacuum grease to the outer top edge of the box or the outer lower edge of the lid. Pour 25 ml of crystallization reagent or common dehydrant into the Sandwich Box. Place the siliconized 9 well glass plate on top of the inverted plastic support. Pipet the sample into one of the nine wells. Add the appropriate crystallization reagent to each drop. Place the cover on the Sandwich Box.

## Sandwich Box Tips

- Apply a thin bead of vacuum grease around a single depression of the glass plate and seal the depression with a plain glass cover slide for long term crystal storage.
- Use a siliconized glass depression plate to test a small amount of sample for solubility with various crystallization reagents.
- Use a Sandwich Box to screen heavy atoms and ligands with crystals.

## 96 Well Plates

The 96 well sitting drop plates offer a variety of drop well configurations and flexibility in a standard microplate footprint. The 8 x 12 reagent wells in 9 mm offset can be filled with automated liquid handling systems or manual, single, and multichannel pipets with a typical reagent fill volume of up to 100  $\mu$ l. The diversity of the various sample drop wells allow for automated and manual pipetting into a variety of well shapes, volumes and geometries. Materials range from optically clear polystyrene to low birefringent polymers that are compatible with UV imaging. The plates can be manually or automatically sealed with optically clear sealing tape or film.

## Plates for Sitting Drop Vapor Diffusion

### 9 well

- HR3-136** Sandwich Box Setup  
Contains: 6 siliconized, 9 well glass plates,  
6 plastic supports,  
6 sandwich boxes with covers

### 24 well

- HR3-159** Cryschem Plate - 40 plate case  
- (round reagent reservoir, large plate footprint)
- Seal with HR3-511 Crystal Clear Sealing Tape (1.88 inch x 43.7 yard roll, with cutter) or HR4-511 Crystal Clear Sealing Tape (1.88 inch x 60 yard roll, without cutter)
- HR1-002** Cryschem M Plate - 50 plate case  
- (round reagent reservoir, small SBS plate footprint)
- Seal with HR3-609 Crystal Clear Sealing Film (100 pack), HR4-521 ClearSeal Film (100 pack) or HR4-506 Crystal Clear Sealing Tape (3 inch x 55 yard roll, without cutter). HR4-508 Crystal Clear Sealing Tape (0.75 inch x 650 inch, with cutter) seals a single row at a time.
- HR3-308** Cryschem S Plate - 50 plate case  
- (square reagent reservoir, small SBS plate footprint)
- Seal with HR3-609 Crystal Clear Sealing Film (100 pack), HR4-521 ClearSeal Film (100 pack) or HR4-506 Crystal Clear Sealing Tape (3 inch x 55 yard roll, without cutter). HR4-508 Crystal Clear Sealing Tape (0.75 inch x 650 inch, with cutter) seals a single row at a time.

# Sitting Drop Vapor Diffusion Crystallization

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Solutions for Crystal Growth

Crystal Growth 101

page 3

**HR3-171** VDX™ Plate with sealant - 40 plate case and HR3-312 Micro-Bridges 400 pack

- Seal with HR3-233 22 mm x 0.22 mm Siliconized circle cover slides 10.0 ounce case (~1,200 slides)

**HR3-114** Intelli-Plate 24-4 - 40 plate case

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

## 48 well

**HR3-180** MRC Maxi 48-Well Crystallization Plate - 40 plate case

**HR8-152** Intelli-Plate 48-2 - 40 plate case

**HR8-156** Intelli-Plate 48-3 - 40 plate case

- Seal 48 well plates with Seal with HR3-609 Crystal Clear Sealing Film (100 pack), HR4-506 Crystal Clear Sealing Tape (3 inch x 55 yard roll, without cutter) or HR4-521 ClearSeal Film™ (100 pack).

## 96 well

**HR3-271** Corning 3773 - 40 plate case  
CrystalEX 96 Well, Conical Bottom

**HR3-115** Corning 3785 - 40 plate case  
CrystalEX 96 Well, Flat Bottom

**HR8-134** Corning 3556 - 40 plate case  
4 µl round drop well, 1 drop well, COC, untreated

**HR8-136** Corning 3551 - 40 plate case  
4 µl conical flat drop well, 1 drop well, COC, treated

**HR8-138** Corning 3552 - 40 plate case  
2 µl round drop well, 3 drop well, COC, untreated

**HR8-140** Corning 3553 - 40 plate case  
2 µl conical flat drop well, 3 drop well, COC, untreated

**HR8-146** Corning 3550 - 40 plate case  
1 µl conical flat drop well, 3 drop well, COC, untreated

**HR8-160** Corning 3557 - 10 plate case  
1 µl conical flat drop well, 5 drop well, PZero

**HR3-190** CrystalQuick™ 96 Well, Greiner 609101 - 40 plate case  
4 µl square drop well, 3 drop well - 40 plate case

**HR3-095G** CrystalQuick™ Plus 96 Well, Greiner 609830 - 40 plate case  
4 µl square drop well, 3 drop well, LBR, hydrophobic

**HR8-149** CrystalQuick™ Plus 96 Well, Greiner 609130 - 40 plate case  
4 µl square drop well, 3 drop well, hydrophobic

**HR3-089** CrystalQuick™ 96 Well, Greiner 609801 - 40 plate case  
4 µl square drop well, 3 drop well, LBR, hydrophobic

**HR3-304** CrystalQuick™ 96 Well, Greiner 609171 - 80 plate case  
4 µl square drop well, 1 drop well, low profile

**HR3-093G** CrystalQuick™ Plus 96 Well, Greiner 609180 - 80 plate case  
4 µl square drop well, 1 drop well, hydrophobic, low profile

**HR3-285** CrystalQuick™ 96 Well, Greiner 609871 - 80 plate case  
4 µl square drop well, 1 drop well, LBR, low profile

**HR3-281** CrystalQuick™ 96 Well, Greiner 609120 - 40 plate case  
2 µl round drop well, 3 drop well

**HR3-091** CrystalQuick™ 96 Well, Greiner 609820 - 40 plate case  
2 µl round drop well, 3 drop well, LBR

**HR3-117** Intelli-Plate 96-2 Low Profile - 80 plate case

**HR3-119** CrystalMation Intelli-Plate 96-3 low-profile - 80 plate case

**HR3-145** Intelli-Plate 96-2 LVR - 40 plate case

**HR3-185** Intelli-Plate 96-3 LVR - 40 plate case

**HR3-299** Intelli-Plate 96-2 Original - 40 plate case

**HR8-172** Intelli-Plate Flat Shelf - 40 plate case

**HR3-164** Intelli-Plate 96-2 Shallow Well - 40 plate case

**HR3-182** Intelli-Plate 96-2 Shallow Well Low Profile - 80 plate case

**HR3-083** Swissci MRC 2 Well Crystallization Plate - 40 plate case

**HR3-107** Swissci MRC 2 Well Crystallization Plate in UVXPO -  
40 plate case

**HR3-206** Swissci 3 Well Low Profile Crystallization Plate - 40 plate case

**HR3-125** Swissci 3 Well Midi Crystallization Plate (Swissci) - 40 plate case

- Seal 96 well plates with HR3-609 Crystal Clear Sealing Film (100 pack), HR4-506 Crystal Clear Sealing Tape (3 inch x 55 yard roll, without cutter) or HR4-521 ClearSeal Film™ (100 pack).

## 384 well

**HR8-058** CrystalEX 384 Well, Flat Bottom, Corning 3775 - 40 plate case

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

## Other Items for Sitting Drop Vapor Diffusion

**HR4-430** Sticky Pad - each

**HR3-510** Dow Corning® Vacuum Grease, 150 gram tube - each

**HR4-124** X-Acto® Gripster Knife - each

Hampton Research

34 Journey

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