

Sitting Drop Vapor Diffusion Crystallization

Crystal Growth 101

The sitting drop vapor diffusion technique is the 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.

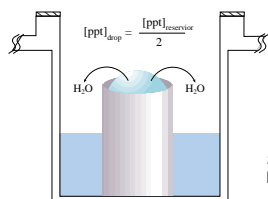


figure 1
Process of vapor diffusion.

Benefits of Sitting Drop Crystallization

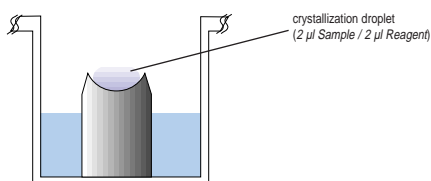
- Allows one to avoid greasing plates.
- Can be cost effective.
- Can be time efficient.
- Often easier when using detergents and hydrophobic reagents.
- Drops can be positioned in a stable sitting position.
- Compatible with gels.

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 microliter drops and the reservoir can hold up to 1.2 microliters of reagent. The Cryschem Plate is sealed with either Clear Sealing Tape or Plain 22 mm Circle or Square Glass Cover Slides. Rows are labeled A-D and columns are labeled 1-6 on the Cryschem Plate.

1. Pipet 0.5 milliliter of crystallization reagent into reservoir A1 of the Cryschem plate. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)
2. Pipet 1 microliter of sample into the post of reservoir A1. (Note: Recommended total drop volume is 1 to 40 microliters)
3. Pipet 1 microliter of reagent from reservoir A1 into the drop in post 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).

figure 2



4. Repeat steps 1 through 3 for the remaining 23 reservoirs.

5. Seal the Cryschem Plate with 2 strips of Clear Sealing Tape (HR4-510).

Cryschem Plate Tips

- Use Crystal Clear Sealing Tape. Other Brands are optically inferior and can turn opaque in the presence of certain crystallization reagents.
- To access a drop and/or reservoir of a Cryschem Plate 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.

Using Micro-Bridges

The Micro-Bridge® is a small bridge (inverted U) manufactured from clear polystyrene and 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 microliter drops. The Micro-Bridge is inserted into the reservoirs of VDX, Linbro, or Costar 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.

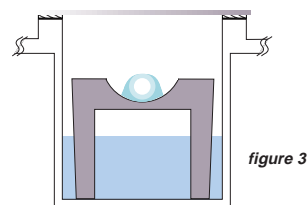


figure 3

1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of a VDX, Linbro, or Costar 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.
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 2 strips of Clear Sealing Tape (HR4-510). The VDX, Linbro, and Costar Plates can also be sealed using sealant and plain glass cover slides.

Micro-Bridge Tips

- Use Crystal Clear Sealing Tape. Other Brands are optically inferior and can turn opaque in the presence of certain crystallization reagents.
- 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

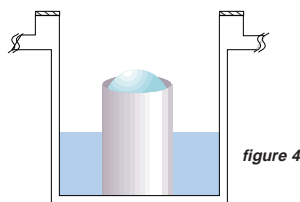
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high vacuum grease.

- 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 nor autoclaved.

Using Glass Sitting Drop Rods

The Glass Sitting Drop Rod™ is a small solid rod manufactured from clear glass which has a smooth, concave depression in the center of the top region of the rod (figure 4). The opposite end of the glass rod is flat. The Glass Sitting Drop Rod can hold up to a 100 microliter drop. The Glass Sitting Drop Rod is inserted into the reservoirs of VDX, Linbro, or Costar plates to perform a sitting drop vapor diffusion experiment. The Glass Sitting Drop Rod can be secured to the bottom of the plate using high vacuum grease or can be left unattached to the bottom of the plate. The Glass Sitting Drop Rod can be removed from the plate for crystal manipulation and observation if desired.



1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of a VDX, Linbro, or Costar Plate. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters; Additional Note: If high vacuum grease will be used to secure the Glass Sitting Drop Rod to the plate, apply the grease to the rod and insert the rod prior to pipetting reagent into the reservoir)
2. Place a clean (blow the Glass Sitting Drop Rod with clean, dry compressed air before use), siliconized Glass Rod into the bottom of reservoir A1 such that the concave depression in the Glass Sitting Drop Rod is facing up.
3. Pipet 2 microliters of sample into the Glass Sitting Drop Rod in reservoir A1. (Note: Recommended total drop volume is 1 to 100 microliters)
4. Pipet 2 microliters of reagent from reservoir A1 into the drop in the Glass Rod 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 2 strips of clear sealing tape.

Glass Sitting Drop Rods Tips

- Use Crystal Clear Sealing Tape. Other Brands are optically inferior.
- To access a drop and/or reservoir of a plate 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.
- Glass Sitting Drop Rods can be removed for crystal seeding, mounting, manipulation, and observation.

- Siliconize Glass Sitting Drop Rods using Aqua Sil™ (HR4-611) or other glass siliconization reagent.
- Glass Sitting Drop Rods may be washed and used over and over again. However, if High Vacuum Grease is used to secure the Glass Sitting Drop Rod to the bottom of the reservoir, we wish you good luck in completely removing the grease from the Glass Sitting Drop Rod.
- Glass Sitting Drop Rods may be autoclaved.

Using the Q Plate & Q Plate II

The Q Plate and Q Plate II are both 24 well plates manufactured from polycarbonate plastic. Each well of the Q Plate contains a two step positioned at 12:00, 3:00, 6:00, and 9:00 at the perimeter of the reservoir. The steps allow an 18 mm or 22 mm circle cover slide to rest on the steps above the bottom of the reservoir. The Q Plate II has a smaller footprint than the Q Plate. Each reservoir of the Q Plate II has a ledge surrounding the perimeter of each reservoir. An 18 mm circle cover slide can be placed on the ledge for sitting drop vapor diffusion experiments. Both plates are sealed with Clear Sealing Tape (HR4-510).

Procedure for Using the Q Plate

1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of the Q Plate. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)
2. Place an 18 mm or 22 mm siliconized cover slide onto the step inside reservoir A1 of the Q Plate. (Note: An 18 mm cover slide rests on the lower step while a 22 mm cover slide rests on the upper step. Since the 18 mm cover slide is closer to the reservoir and the space between the slide and the reservoir is larger, vapor equilibration occurs at a higher rate than when using 22 mm cover slides that sit higher and has a smaller space between the slide and the reservoir wall).
3. Pipet 2 microliters of sample onto the siliconized cover slide in reservoir A1. (Note: Recommended total drop volume is 1 to 20 microliters)
4. Pipet 2 microliters of reagent from reservoir A1 into the drop on the siliconized slide in reservoir 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 4 for the remaining 23 reservoirs.
6. Seal the Q Plate plate with 3 strips of Clear Sealing Tape (HR4-510).

Procedure for Using the Q Plate II

1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of the Q Plate II. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)
2. Place an 18 mm siliconized cover slide onto the ledge inside reservoir A1 of the Q Plate II.
3. Pipet 2 microliters of sample onto the siliconized cover slide in reservoir A1. (Note: Recommended total drop volume is 1 to 20 microliters)
4. Pipet 2 microliters of reagent from reservoir A1 into the drop on the siliconized

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slide in reservoir 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 4 for the remaining 23 reservoirs.

6. Seal the Q Plate II plate with 2 strips of Clear Sealing Tape (HR4-510).

Q Plate & Q Plate II Tips

- Use Crystal Clear Sealing Tape. Other Brands are optically inferior and the adhesive will turn opaque with certain crystallization reagents.
- To access a drop and/or reservoir of a Q Plate or Q Plate II 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.
- One can pipet multiple drops onto the cover slide. This technique is often useful when screening additives since one can use the same reservoir with multiple drops with each drop containing a different additive. This technique can also be used to screen different drop sizes and ratios versus the same reservoir. Use care not to avoid mixing the drops during pipetting, plate transport, and plate viewing.
- The Q Plate can also be used for hanging drop and sandwich drop vapor diffusion experiments. The Q Plate II can also be used for hanging drop vapor diffusion experiments.
- Use care when transporting and viewing Q Plate and Q Plate II's. A bump to the plate can toss the cover slide out of position or onto the tape. In very dry, high static environments one may prefer to treat the plates and slides with a static removing device to prevent the glass slides from "jumping" onto the tape.

CrystalClear Strips

CrystalClear Strips consist of a plastic frame and 12 polystyrene strips with a total experiment capacity of 96 per plate. Each strip contains 8 reservoirs and platforms. Typical reservoir volumes are 100 microliters. The smooth, concave depression on the platform above the reservoir can hold up to a 10 microliter drop. The CrystalClear Strip is sealed with Clear Sealing Tape.

Pipet 100 microliters of crystallization reagent in each of the 96 reservoirs. Pipet 1 microliter of sample into the depression on the ledge of the first reservoir A1. Pipet 1 microliter of reagent from reservoir A1 into the drop on the ledge above reservoir A1. Repeat steps 2 and 3 for the remaining 95 reservoirs and wells. Seal the CrystalClear Strips with Clear Sealing Tape (HR4-510).

CrystalClear Strips Tips

- The strips are available with and without a concave depression for drop placement.
- While pipetting reagents into the reservoirs, place a clean pipet tip into the first empty reservoir. Move the clean tip ahead one empty reservoir with each reagent addition. This will help one keep track of their pipetting position in the plate.

Sandwich Box

The Sandwich Box consists of a square polystyrene box, a petri dish half, 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 petri dish half, 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 milliliters of crystallization reagent or common dehydrant into the Sandwich Box. Place the siliconized 9 well glass plate on top of the inverted petri dish. Pipet the sample into one of the 9 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 small amount of sample for solubility with various crystallization reagents.

The Zeelan SetUp

The Zeelan SetUp in a sandwich box setup for hanging drop vapor diffusion crystallization. The Zeelan SetUp consists of a polystyrene box and cover, and two polycarbonate 12 well plates. The polystyrene outer box and cover are used to store and protect the experiment. The actual experiment is performed in the polycarbonate inner plate on siliconized glass cover slides. One advantage of using the Zeelan SetUp is that polycarbonate does not birefringe. This makes viewing and scoring crystallization drops with polarizing optics easier since there is no interfering birefringence from the plastic.

Pipet a bead of vacuum grease about each reservoir in the 12 well polycarbonate plate. Pipet the crystallization reagent into reservoir 1. Pipet 1 microliter of reagent from reservoir 1 onto a clean, siliconized 22 mm circle cover slide. Pipet 1 microliter of sample into the sample drop on the cover slide. Carefully invert the slide and seal over reservoir one. Repeat steps 2 through 5 for the remaining 11 reservoirs. Place the completed plate inside the Zeelan Box. Repeat steps 1 through 6 for the second Zeelan Plate and place the second plate alongside the first, inside the Zeelan Box.

Zeelan SetUp Tips

- Remove the Zeelan Plate from the Zeelan Box for drop viewing.
- Zeelan Boxes can be used over and over again.

Technical Support

Inquiries regarding the sitting drop crystallization method, interpretation of screen results, optimization strategies 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.