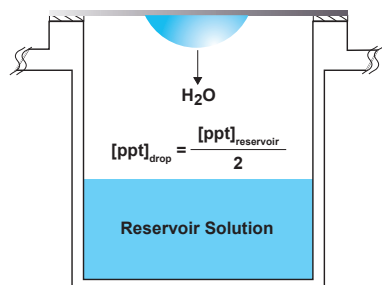


# Hanging Drop Vapor Diffusion Crystallization

The hanging 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 equilibrium 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 Hanging Drop Crystallization

- Can be cost effective.
- Sample and reagents in contact with a siliconized glass surface.
- Easy access to crystals.
- Can perform multiple drops (experiments) with a single reservoir.

## Using the VDX Plate for Hanging Drop Vapor Diffusion

The VDX Plate is a 24 well plate manufactured from clear polystyrene. The VDX Plate is typically sealed with High Vacuum Grease and Siliconized 22 mm Circle or Square Glass Cover Slides. Rows of the plate are labeled A-D and columns are labeled 1-6 on the VDX Plate.

## Applying Sealant to a VDX Plate

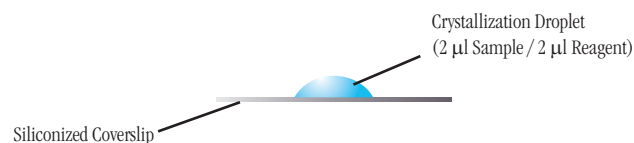
1. Apply a bead of High Vacuum Grease along the top edge of the raised reservoir A1 of the VDX Plate. It is recommended that one apply the high vacuum grease prior to pipetting the reagent. Create a circular bead on the upper edge of the reservoir. Do not complete the circle. Leave a 2 mm opening between the start and finish of the circular bead. Leave a 2 mm opening between the start and finish of the circular bead as this will allow air pressure to escape when sealing the reservoir with a cover slide. For convenience, time savings and a consistent seal, the VDX Plate is also available with sealant.

2. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of the VDX Plate. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)

3. Clean a Siliconized 22 mm Circle or Square Cover Slide by wiping the cover slide with lens paper and blowing the cover slide with clean, dry com-

pressed air. Pipet 2 microliter of sample into the center of a Siliconized 22 mm Circle or Square Cover Slide. (Note: Recommended total drop volume is 2 to 20 microliters)

**Figure 2**



4. Pipet 2 microliter of reagent from reservoir A1 into the drop on the cover slide containing the sample. (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 homogeneous drop and consistency drop to drop. Proponents of not mixing the drop simply pipet the reagent into the sample with no further mixing. Not mixing allows for the sample and reagent to mix by liquid diffusion).

5. Holding the cover slide with forceps, the Pen-Vac, or on the edge between your thumb and forefinger, carefully yet without delay invert the cover slide so the drop is hanging from the cover slide.

6. Position the cover slide onto the bead of grease on reservoir A1. Gently press the slide down onto the grease and twist the slide 45° to ensure a complete seal.

7. Repeat for reservoirs 2 (A2) through 24 (D6).

## Hanging Drop Tips

- Note the VDX Plate has a raised cover to protect the cover slides during transport and storage.
- To access a drop and/or reservoir simply grasp the edge of the cover slide with forceps or fingertips, twist and pull gently while holding the plate steady. The Thick (0.96 mm) cover slides resist heavy handling better than the regular (0.22) cover slides.
- VDX Plates can be stacked for convenient storage.
- 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.
- Label plates with protein name, experiment, drop & reservoir volumes, and date.
- Use the Glass Cover Slide Gizmo Dispenser for fast and easy handling of siliconized cover slides. Or try the Cover Slide Vacuum Gadget (HR8-098) or Pen-Vac (HR3-251) for holding and manipulating cover slides.

# Hanging Drop Vapor Diffusion Crystallization

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## Plates for Hanging Drop Vapor Diffusion

### **24 well, use 22 mm cover slide**

**HR3-140** VDX™ Plate without sealant - 100 plate case

**HR3-170** VDX™ Plate with sealant - 100 plate case

### **24 well, use 18 mm cover slide**

**HR3-108** VDXm™ Plate without sealant - 50 plate case

**HR3-306** VDXm™ Plate with sealant - 50 plate case

**HR3-200** 24 Well ComboPlate without sealant, Greiner 662150 - 24 plate case

### **48 well, use 12 mm cover slide**

**HR3-275** VDX48 Plate with sealant - 50 plate case

### **96 well**

**HR3-197** 96 Well Hanging Drop Vapor Diffusion Plate - 100 pack

**HR3-187** Hanging Drop Crystallization Plate (Swissci) - 10 plate case

## Siliconized Cover Slides for Hanging Drop Vapor Diffusion

**HR3-277** 12 mm x 0.22 mm Siliconized circle cover slides  
0.5 ounce pack (~240 slides)

**HR8-090** 12mm x 0.96mm Thick Siliconized circle cover slides  
10.0 ounce case (~1,060 slides)

**HR3-241** 18 mm x 0.22 mm Siliconized circle cover slides  
5.0 ounce case (~1,000 slides)

**HR3-517** 18mm x 0.96mm Thick Siliconized circle cover slides  
10 oz case (~450 slides)

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

**HR3-249** 22mm x 0.96mm Thick Siliconized circle cover slides  
30.0 ounce case (~750 slides)

**HR3-217** 22 mm x 0.22 mm Siliconized square cover slides  
10.0 ounce case (~1,000 slides)

**HR3-225** 22mm x 0.96mm Thick Siliconized square cover slides  
40.0 ounce case (~750 slides)

## Sealing Grease

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

**HR3-508** Dow Corning® 7 Release Compound Grease, 150 gram tube - each

## Other Items for Hanging Drop Vapor Diffusion

**HR4-430** Sticky Pad - each

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