Gels are a very efficient media for growing macromolecular crystals. Silica gels in particular have the advantage in that they are stable, usable over a wide range of temperature (0-60°C), and are compatible with a wide variety of precipitants and additives used for crystal growth. The Hampton Research Silica Hydrogel™ kit provides you with the material required to make gels at room temperature which polymerize rapidly and form a porous matrix with a pH of 7.0. The neutral pH allows one to use the gel with minimal influence on the pH of the precipitant.

Suggested Uses
- To reduce excessive nucleation
- To reduce excessive precipitate
- To grow fewer, larger crystals
- To change the morphology of the crystal
- To slow the crystal nucleation and/or growth of crystals and to alter the kinetics of the experiment.

These effects and their extent will vary with each macromolecule and precipitant/crystallization system. It should be noted that in some instances the use of a gel for crystal growth may have a detrimental effect under the selected crystallization conditions. One should seek to optimize conditions for gel growth (temperature, precipitant type/concentration, etc.).

Sample Preparation
The sample should be as pure as is practically possible (> 95%) and free of amorphous and particulate material. Remove amorphous material by centrifugation or microfiltration prior to use.

Recommended sample concentration is 5 to 25 mg/ml. The sample should be free of any unnecessary additives. Ideally, the initial screen should be performed with a sample which has been dialyzed against water. Ligands, ions, reducing agents, or other additives may be present as required by the sample for solubility, stability, or activity.

Using the Silica Hydrogel - Sitting Drop Methodology
1. The gel is created by mixing equal parts of the sodium silicate solution (Green Top Tube) with dilute acetic acid (Blue Top Tube). This can be accomplished by placing the entire 0.5 ml of the 2.8% (v/v) sodium silicate solution (Green Top Tube) into the dilute acetic acid solution (Blue Top Tube), or mix any custom volume you might require in a separate container as long as the ratio of sodium silicate to acetic acids is 1:1 (i.e. 200 μl plus 200 μl). **Do not add the acetic acid into the sodium silicate!** It is very important that the sodium silicate be added to the acetic acid solution. Figure 1.

2. Mix by vortexing for 30 seconds. This is your gel stock. It will polymerize in 5 to 10 minutes. The gel will have a neutral pH of 7.0.

3. Pipet 1 - 5 μl of the gel onto a Micro-Bridge or the post of the Cryschem plate. Allow to polymerize. This will require approximately 5 to 10 minutes. Allow one hour for a complete cure. A small amount of water may appear on the surface of the gel after cure. Remove this water with a Paper Wick or small piece of filter paper. Seal the reservoir with a cover slide or Crystal Clear Sealing Tape while you are waiting for the gel to polymerize. The gel will dehydrate if left open.

4. Add 1 - 5 μl of the precipitant you intend to place in the reservoir to the TOP (not the side) of the gel drop. Figure 2. Allow this precipitant to be completely absorbed into the gel. Complete absorption will require one hour for dilute precipitants and as long as eight hours for high concentrations of PEG and MPD. Again, keep the reservoir covered during this time.

5. Place 2 - 10 μl of your protein/peptide/small molecule solution onto the gel. Allow to soak in completely. This will require approximately one hour. Keep the reservoir covered during this time.

6. Place 0.7 to 1.0 ml of precipitant into the reservoir and seal the plate with Crystal Clear Sealing Tape or a cover slide.

Using the Silica Hydrogel - Liquid / Gel Diffusion
See Sitting Drop Vapor Diffusion for inscription on preparing the gel solution.

1. Place 2 - 20 μl of gel into a small culture tube. Allow the gel solution to polymerize and cure for approximately one hour.

2. Add 2 - 20 μl of protein to the polymerized gel. Allow the material to be completely absorbed. This should require approximately one hour.
3. Add 4 - 40 μl of precipitant to the top of the gel after the protein has been completely absorbed. Seal with paraffin or wax. See Figure 3 below.

![Figure 3](Image)

**Liquid/Gel Diffusion**

See Sitting Drop Vapor Diffusion for instructions on preparing the gel solution.

1. Using a capillary or caraway tube (Fisher Scientific part number 02-668-35) place a small amount of gel into the center of the tube. Use sufficient gel to create a plug. The length of the plug should be approximately 2 mm. Longer plugs will slow diffusion between precipitant and sample but will also dilute your sample as it diffuses. Allow the gel to polymerize.

2. Place 1 - 20 μl of your sample on one side of the gel plug using a thin tipped sequencing gel loading pipet tip.

3. Place 1 - 20 μl of your precipitant on the opposite side of the gel.

4. Seal the ends of the tube with paraffin, mounting clay, or wax.

**Examine the Drop**

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the screen. Record all observations and be particularly careful to scan the focal plane for small crystals. Observe the drops once each day for the first week, then once a week thereafter. Records should indicate whether the drop is clear, contains precipitate, and or crystals. It is helpful to describe the drop contents using descriptive terms. Adding magnitude is also helpful. Example: 4+ yellow/brown fine precipitate, 2+ small bipyramid crystals, clear drop, 3+ needle shaped crystals in 1+ white precipitate. One may also employ a standard numerical scoring scheme (Clear = 0, Precipitate = 1, Crystal = 10, etc). Figure 5 shows typical examples of what one might observe in a crystallization experiment.

**Interpretation of the Results**

1. Fewer nucleation sites.

2. An increase in the amount of time required to see crystals using microscopy (i.e. slower growth rate).

3. In some cases, different habit, or morphology.

4. In some cases, reduced sedimentation.

5. In some cases, larger, fewer crystals.

**Optimization**

1. Change your protein concentration. If there are too many nucleation sites, reduce protein concentration. No nucleation, increase protein concentration. Be patient as the equilibration will require up to 4 to 8 times longer than identical conditions with out the gel. One may alter these growth times by increasing the sample concentration/precipitant concentration to increase the rate of growth and decreasing the value of these parameters to slow growth.

2. Alter your sample/gel/precipitant ratio.

3. Consider the influence of other variables such as temperature, precipitant type & concentration, the use of ligands or metals, detergents, and the many other variables which could influence crystal growth.

**References and Readings**


Related Products
HR3-158 Cryschem Plate - 24 plate case
HR3-160 Cryschem Plate - 100 plate case
HR3-211 22 mm Plain Square cover slide- 1 oz case (~100 slides)
HR3-213 22 mm Plain Square cover slide- 10 oz case (~1,000 slides)
HR3-215 22 mm Siliconized Square cover slide - 1 oz case (~100 slides)
HR3-217 22 mm Siliconized Square cover slide - 10 oz case (~1,000 slides)
HR3-227 22 mm Plain Circle cover slides - 1 oz case (~120 slides)
HR3-229 22 mm Plain Circle cover slides - 10 oz case (~1,200 slides)
HR3-231 22 mm Siliconized Circle cover slides - 1 oz pack (~100 slides)
HR3-233 22 mm Siliconized Circle cover slides - 10 oz pack (~1,000 slides)
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
HR4-110 Paper Wicks 25 mm - Assorted (XXX Fine - X Fine) - 200 wicks
HR4-122 Paper Wicks 25 mm - XXX Fine - 200 wicks
HR4-112 Paper Wicks 25 mm - XX Fine - 200 wicks
HR4-114 Paper Wicks 25 mm - X Fine - 200 wicks
HR4-116 Paper Wicks 25 mm - Fine - 200 wicks
HR4-118 Paper Wicks 25 mm - Medium - 200 wicks
HR4-120 Paper Wicks 25 mm - Coarse - 200 wicks
HR4-211 Paper Wicks 55 mm - X Fine Long - 100 wicks
HR4-213 Paper Wicks 55 mm - Medium Long - 100 wicks
HR4-215 Paper Wicks 55 mm - Coarse Long - 100 wicks
HR4-310 Red Sticky Wax - 80 wax ropes
HR4-312 Beeswax Sticks - 7 sticks
HR4-328 Capillary Wax - 40 gram pack (approximate)
HR4-326 Four Color Mounting Clay - 16 oz pack (4 oz each, blue / yellow / red / green)
HR4-342 Wax Pen - 1 each
HR4-344 Wax Pen Replacement Tip- 3 pack
HR4-334 Capillary Cutting Stone - 1 each
HR6-104 0.1 mm Glass #50 Capillaries - 25 pack
HR6-106 0.2 mm Glass #50 Capillaries - 25 pack
HR6-108 0.3 mm Glass #50 Capillaries - 25 pack
HR6-110 0.4 mm Glass #50 Capillaries - 25 pack
HR6-112 0.5 mm Glass #50 Capillaries - 25 pack
HR6-114 0.6 mm Glass #50 Capillaries - 25 pack
HR6-116 0.7 mm Glass #50 Capillaries - 25 pack
HR6-118 0.8 mm Glass #50 Capillaries - 25 pack
HR6-120 0.9 mm Glass #50 Capillaries - 25 pack
HR6-122 1.0 mm Glass #50 Capillaries - 25 pack
HR6-124 1.5 mm Glass #50 Capillaries - 25 pack
HR6-126 2.0 mm Glass #50 Capillaries - 25 pack
HR6-128 0.1 mm Quartz Capillaries - 25 pack
HR6-130 0.2 mm Quartz Capillaries - 25 pack
HR6-132 0.3 mm Quartz Capillaries - 25 pack
HR6-134 0.4 mm Quartz Capillaries - 25 pack
HR6-136 0.5 mm Quartz Capillaries - 25 pack
HR6-138 0.6 mm Quartz Capillaries - 25 pack
HR6-140 0.7 mm Quartz Capillaries - 25 pack
HR6-142 0.8 mm Quartz Capillaries - 25 pack
HR6-144 0.9 mm Quartz Capillaries - 25 pack
HR6-146 1.0 mm Quartz Capillaries - 25 pack
HR6-148 1.5 mm Quartz Capillaries - 25 pack
HR6-150 2.0 mm Quartz Capillaries - 25 pack
HR6-151 2.5 mm Quartz Capillaries - 25 pack
HR6-152 0.1 mm Special Glass 10 Capillaries - 25 pack
HR6-154 0.2 mm Special Glass 10 Capillaries - 25 pack
HR6-156 0.3 mm Special Glass 10 Capillaries - 25 pack
HR6-158 0.4 mm Special Glass 10 Capillaries - 25 pack
HR6-160 0.5 mm Special Glass 10 Capillaries - 25 pack
HR6-162 0.6 mm Special Glass 10 Capillaries - 25 pack
HR6-164 0.7 mm Special Glass 10 Capillaries - 25 pack
HR6-166 0.8 mm Special Glass 10 Capillaries - 25 pack
HR6-168 0.9 mm Special Glass 10 Capillaries - 25 pack
HR6-170 1.0 mm Special Glass 10 Capillaries - 25 pack
HR6-172 1.5 mm Special Glass 10 Capillaries - 25 pack
Technical Support

Inquiries regarding the Silica Hydrogel 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.