LM Agarose™

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Solutions for Crystal Growth

Material Description

Low melting (LM) Agaroses are the result of a derivatization process by organic synthesis. Essentially, the process generates methoxylate groups from the basic Agarose structure. The main properties of these Agaroses are their low melting and gelling temperatures when compared with standard Agaroses. LM Agaroses have lower gel strength than standard Agaroses yet can be handled easily. LM Agaroses have higher clarity (gel transparency) than gels of standard Agaroses. LM Agaroses have great sieving capacity. The gelling temperature of LM Agaroses is 24 to 28 degrees Celsius.

Agarose is a neutral polysaccharide extracted from the cellular walls of Rhodophyceae algae belonging to the genera Gelidium, Gelidiella, Pterocladia, Gracilaria, and Ahnfeltia, also known as agarophyte seaweed. The structure of the polysaccharide is that of a galactan, formed by linking agarobioses by links 1-3, 1-4. This chemical structure gives Agaroses the capacity to form strong gels even at low temperatures. The gels have a macroreticular structure with a very open mesh which can be adjusted simply by varying the concentration of the Agarose. The macroreticule structure of the Agarose gel is formed by hydrogen bonds, which makes the gel reversible, transforming the gel into a solution by heating. The hysteresis (difference between gelling and melting temperature) is greater than any other hydrocolloid. The absence of ionic groups makes the gel a neutral structure. With no interaction, macromolecules can migrate through the gel mesh, making the gel an efficient sieve for biological macromolecules.

The LM Agarose offered by Hampton Research is 100% pure, does not contain any additives, does not contain ligation inhibitors and is free of DNAses and RNAses. Hampton Research LM Agarose is clearer than other Agaroses and also has a higher gel strength.

Why Agarose Gels for Crystallization?

Agarose gels can reduce convection and regulate the diffusion of biological macromolecules, as well as crystallization reagents and can influence the crystallization of biological macromolecules. LM Agaroses are especially useful for crystallization as their gelling point minimizes thermal shock and protein damage compared to standard Agaroses with higher melting and gelling points.

Agarose as well as silica and polyacrylamide gels have been used for some time to obtain high quality crystals of small molecules as well as biological macromolecules. Crystal growth in a gel can prevent the onset of convection. Crystals grown in gels are suspended in their mother liquor within the gel network. Crystal nucleating and growing in a gel do not sediment as they are sustained in the gel network. Gels promote crystal growth in three dimensions and can provide protection during transport, mounting and soaking. Generally, with Agarose gels the number of crystals (in a particular setup) increases by increasing the gel concentration.

Formulating LM Agarose Gels for Crystallization

Agarose gels are typically supplied as powders that can be dissolved in water. A 2% Agarose gel stock solution is quite typical and concentrations higher than 2% are difficult to formulate. Crystal nucleation and growth is typically performed with a final gel concentration of 0.1 to 0.3% Agarose in water. It is convenient for formulate a 2% w/v LM Agarose working stock from which dilutions can be made to create final LM Agarose gel concentrations of 0.1 to 0.3% Agarose in the presence of crystallization reagents and sample (protein, peptide, nucleic acid).

A general procedure for making a 2% w/v LM Agarose gel stock follows.
Warning: Physical injury hazards are possible when using hot surfaces and liquids. Use care and appropriate safety protection when handling boiling liquids. Use caution when using a microwave to dissolve the Agarose. Agarose can become superheated and boil suddenly when removed from the microwave.

Add progressively more amounts of a total mass of 0.2 grams of LM Agarose to a final volume of 10 milliliters of sterile filtered deionized water. Adding gel to water minimizes clumping and sticking of the gel to the mixing container. Add the gel to the solution and avoid getting the gel on the sides of the container to prevent clumping. One may use a glass stir rod to break clumps, but one must be careful to not remove Agarose or solution from the mixture by having the material stick to the stir rod (this will alter the final gel concentration). Raise the temperature between 65 and 100 degrees Celsius in a water bath and swirl the solution or use a magnetic stir bar and plate to mix the solution until it becomes homogeneous and as clear as water. One may loosely cover the container with a cap or sealing film but be sure to allow for venting of pressure inside the vessel as the temperature increases inside the container. Maintain the temperature between 35 and 45 degrees Celsius to keep the gel in a liquid state for crystallization experiments or allow the gel to cool to 28 degrees Celsius (as low as 4 degrees Celsius; temperatures below 4 degrees Celsius can damage the Agarose) for gelling. A 2% LM Agarose stock can be stored at 4 degrees Celsius to minimize the risk of microbial growth.

Formulating an LM Agarose stock using sterile filtered deionized water from a sterile container directly into a sterile glass container with heating done carefully in a microwave or water bath with subsequent storage of the gel stock or aliquots of the gel stock at 4 degrees Celsius will help to maintain a clean, microbial free LM Agarose. Use of sterile plastic bottles or tubes for storage of formulated stocks or aliquots will help to maintain the sterility of the gel. Hampton Research LM Agarose is 100% pure and free of microbes as well as DNAse and RNAse. Gelled LM Agaroses can be media for microbial growth so to avoid microbial contamination it is best to create and observe a clean work area during the formulation and storage of LM Agarose gels as well as during the set up of crystallization experiments involving gels.

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Crystallization in Agarose Gels

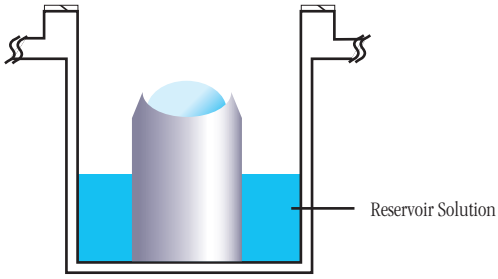
A general discussion of crystallization in LM Agarose gels follows. The following should be interpreted as a general guideline and starting point for using gels in protein crystallization experiments. Creativity in experimental design is encouraged when using gels in order to explore and exploit the unique attributes of gels.

A 2% working stock of Agarose gel can be used to create vapor diffusion, microdialysis, batch, or liquid gel diffusion crystallization experiments. Agarose gels are compatible with a wide variety of crystallization methods, hardware and reagents.

Vapor diffusion and LM Agarose Gels

Protein and reagents may be added to the LM Agarose solution before gelation as the protein and most popular crystallization reagents do not perturb the gelation process. Pipet the appropriate crystallization reagent into the plate reservoir. See Figure 1 below. Pipet LM Agarose gel solution, protein and reagent into a small vial maintained above the gelling temperature (35 to 45 degrees Celsius for LM Agarose), gently mix using a pipet and place the desired drop volume on the appropriate vapor diffusion surface (cover slide, post, ledge, etc) and seal the experiment. One may explore varying drop ratios of gel, protein and reagent concentrations. Recommended final gel concentration is 0.1-0.4%. For example, a 10 microliter drop might contain 2 microliters of gel, 3 microliters of reagent and 5 microliters of protein.

Figure 1
Cross section of a reservoir in the Cryschem plate.

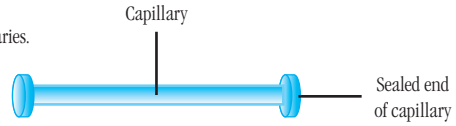


Additional Comment: The gel is typically not added to the reservoir. However, the gel be used in the reservoir as a way to control the rate of vapor diffusion. A gelled reservoir will slow the rate of vapor diffusion between the reservoir and the drop. However, the use of oil layered on the reservoir may be a more convenient and a cost effective alternative to using gel in the reservoir.

Microbatch and LM Agarose Gels

Protein and reagents may be added to the LM Agarose solution before gelation as the protein and most popular crystallization reagents do not perturb the gelation process. Pipet LM Agarose gel solution, protein and reagent into a small vial maintained above the gelling temperature (35 to 45 degrees Celsius for LM Agarose), gently mix using a pipet and place the desired volume into a glass or quartz capillary and seal the ends of the capillary. See Figure 2 below. One may explore varying ratios of gel, protein and reagent concentrations. Recommended final gel concentration is 0.1-0.4%. For example, a 10 microliter drop might contain 2 microliters of gel, 3 microliters of reagent and 5 microliters of protein. One may also set gel microbatch experiment under oil using various microbatch plates.

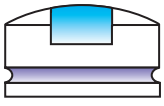
Figure 2
Microbatch in capillaries.



Microdialysis and LM Agarose Gels

Protein and reagents may be added to the LM Agarose solution before gelation as the protein and most popular crystallization reagents do not perturb the gelation process. Pipet LM Agarose gel solution, protein and reagent into a small vial maintained above the gelling temperature (35- 45 degrees Celsius for LM Agarose), gently mix using a pipet and place the desired volume into a microdialysis button and apply the dialysis membrane and O-ring as for a typical microdialysis experiment. See Figure 3 below. Place the microdialysis button or cell into a reservoir of equilibrant solution. One may explore varying ratios of gel, protein and reagent concentrations. Recommended final gel concentration is 0.1-0.4%. For example, a 10 microliter experiment might contain 2 microliters of gel, 3 microliters of reagent and 5 microliters of protein.

Figure 3
Gel crystallization by Microdialysis



Counter Diffusion and LM Agarose Gels

The combination of a long diffusion pathway (capillary, tube, or tubing) and a gel can be combined to create a novel crystallization vessel with unique diffusion and equilibration kinetics which may be exploited as a unique crystallization variable for screening and/or optimization. In a Counter Diffusion experiment a concentration wave travels along the capillary creating a gradient of sample and reagent concentration. Counter Diffusion may be explained as one solution/

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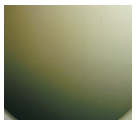
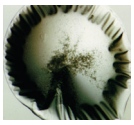
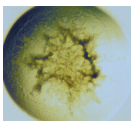
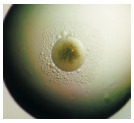
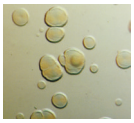
Figure 6
Typical observations in a crystallization experiment

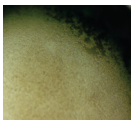

Clear Drop


Skin / Precipitate

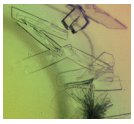

Precipitate


Precipitate / Phase

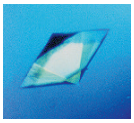

Quasi Crystals


Microcrystals


Needle Cluster

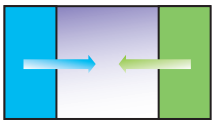

Plates


Rod Cluster


Single Crystal

media placed in front of another solution/media (free interface diffusion), a gel plug separating two solutions (sample and reagent) or some other physical arrangements of the solution and gel media to avoid or minimize convection and construct a counter diffusive system. See Figure 4 below.

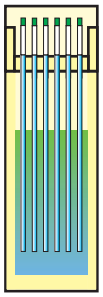
Figure 4
Liquid / Gel / Liquid Diffusion.



Counter Diffusion Example: Prepare a 1.5% LM Agarose solution in water. Pour or pipet the Agarose solution into a Petri dish bottom or other suitable container (or use a Granada Crystallization Box (GCB) - HR3-194). See Figure 5 on the following page. Allow the gel to solidify. Pipet the protein into a capillary so the protein solution is approximately 5 centimeters into the capillary. Seal the end of the capillary using clay (or your favorite sealant) which features the air gap above the sample. The end of the capillary butted with sample is then pierced into the solidified gel. The appropriate crystallization reagent is then pipetted onto the gel surface. Reagent will diffuse into and through the gel into the sample inside the capillary.

Counter-Diffusion Variation Example: Mix gel solution and sample, then pipet this mixture into the open end of a capillary being careful to avoid air bubbles. At the opposite end of the capillary add the crystallization reagent. Work carefully to create a clean interface between the sample/gel and the reagent. Seal ends of the capillary with sealant (wax, clay, nail polish, epoxy, etc.). Note: One may use sequencing gel pipet tips for sample loading. Load reagents at one end. Maintain liquid meniscus at opening edge of capillary. Now pipet gel/sample into the capillary, tilting the capillary to allow the reagent and gel/sample to move into the capillary without air gaps or bubbles. Seal the capillary. Practice with water and gel or dye (food coloring) and gel to master the technique then try it with the sample to minimize personal stress and sample waste.

Figure 5
Counter diffusion example.



Examine The Drop

Carefully examine the drops under a stereo microscope (10 to 100x magnification) immediately after setting up the experiment. 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 there after. 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 6 shows typical examples of what one might observe in a crystallization experiment.

Technical Specifications

(Please refer to the COA for complete specification)

- pH in solution 6.7
- pH in gel 5.96
- Gelling temperature 24-28 degrees Celsius
- Solution Temperature 35 to 45 degrees Celsius
- Melting temperature > 64 degrees Celsius
- DNAse and RNAse None detected
- Recommended stock concentration: 2% w/v

Recommended gel concentration for crystallization: 0.1 to 0.4% for traditional crystallization (vapor diffusion, batch and microdialysis); 1 to 1.5% for counter-diffusion.

References and Readings

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11. Chayen, N.E., "The Role of Oil in Macromolecular Crystallisation" Structure 5 (1997), 1269-1274.

Technical Support

Inquiries regarding the LM Agarose for Crystallization 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 4:30 p.m. USA Pacific Standard Time.

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