Lonza Rockland, Inc.
www.lonza.com
biotechserv@lonza.com
Tech Service: 800-521-0390
Customer Service: 800-638-8174
Document # 18842-1007-02
Rockland, ME 04841 USA

SeaPrep® Agarose

An ultralow gelling, soft agarose

Introduction

SeaPrep® Agarose is a specialty "soft agarose" product with ultralow gelling and melting temperature properties which make it valuable for many techniques involving biomolecules. SeaPrep® Agarose is an excellent medium for hybridoma cloning in soft, sparkling clear gels. (The clear sol may be stored sterile, almost indefinitely, in a 37ºC incubator.) SeaPrep® Agarose gels below 17ºC and then may be melted at 40ºC-50ºC. The extended ultralow working temperature range permits great latitude and convenience for handling of heat-labile biomolecules.

At concentrations of 0.8%-1.5%, the soft gel texture makes SeaPrep® Agarose a suitable suspension medium for cells and facilitates their recovery by gentle mechanical disruption for subsequent growth in liquid media. Growth factors can be added to SeaPrep® Agarose along with other nutrients to produce a "defined" medium. Prepurchase samples of SeaPrep® Agarose are available for evaluation of compatibility with specific cell lines.

Analytical Specifications

Gelling temp. (0.8%)	8ºC-17ºC
Melting temp. (1.0%)	≤ 50ºC
Moisture:	≤ 10%
Sulfate:	≤ 0.10%
EEO($-m_r$):	≤ 0.05
Gel strength (2%):	≥ 75g/cm^2

Properties

SeaPrep® Agarose is prepared by the controlled introduction of hydroxyethyl groups to the agarose molecule (Figure 1). The reduction in gelling temperature from the natural agarose value of approximately 36ºC is a function of the degree of hydroxyethylation (Figure 1).

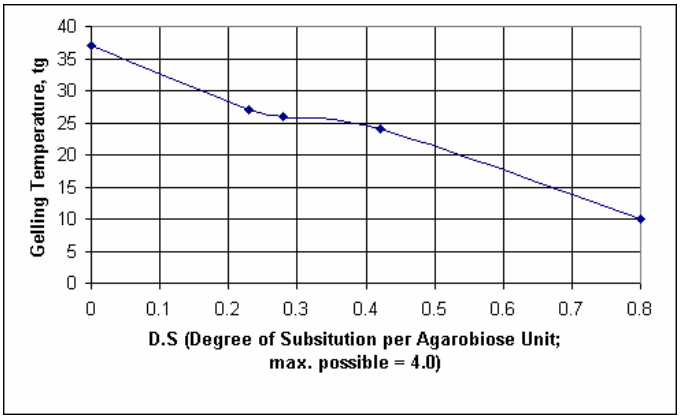

Figure 1. Gelling temperature as a function of the degree of hydroxyethylation.

Two consequences of the hydroxyethylation of agarose are a reduction in gel strength and an increase of gel clarity. The relationship between gel strength and gelling temperature is illustrated in Figure 2.

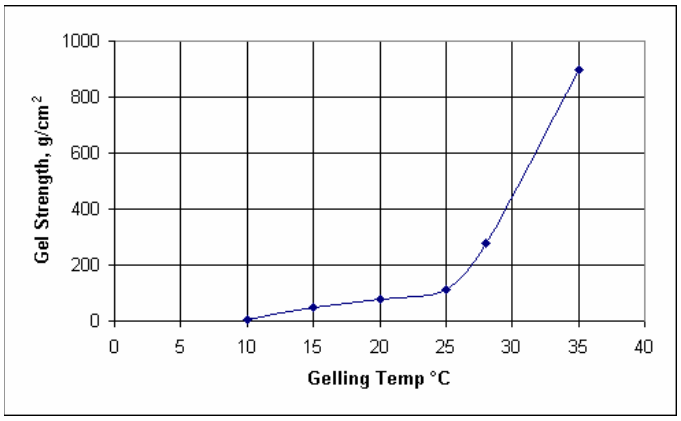

Figure 2. Relationship between gelling temperature and gel strength.

SeaPrep® Agarose falls near the bottom of the curve in Figure 2, with a 1% gelling temperature below about 20ºC.

Gelling temperature, melting temperature, and gel strength all vary considerably with concentration of SeaPrep® Agarose in a gel (Figures 3 and 4). In Figure 4, the effect of concentration is particularly pronounced at concentrations below 1.5%. SeaPrep® Agarose should be used above 2% if a usable gel structure is desired. At higher levels, the remarkable clarity of SeaPrep® Agarose remains, allowing the investigator to easily view samples, such as cells, within gels. In horizontal electrophoretic applications, concentrations above 2% are recommended.

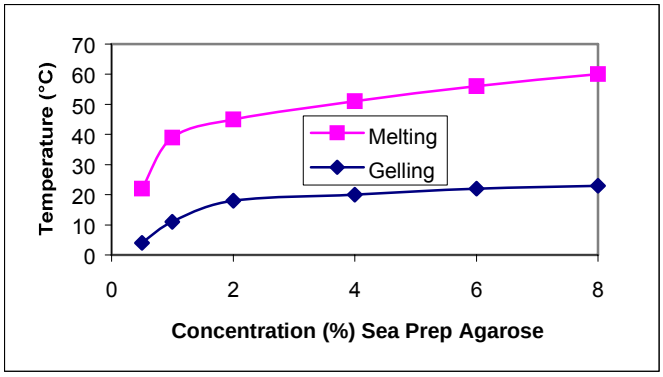

Figure 3. Relationship between gelling/melting temperature and gel concentration of SeaPrep® Agarose.

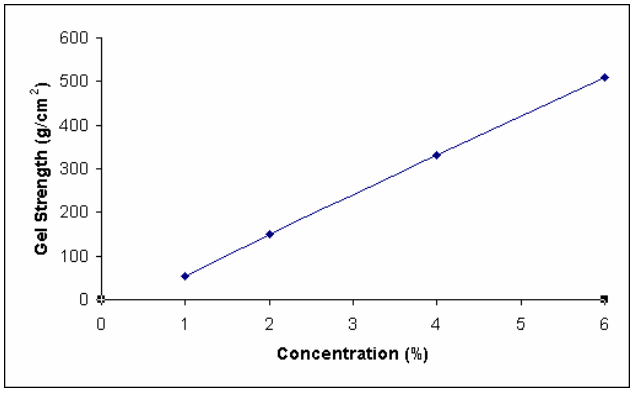

Figure 4. Relationship between gel strength and gel concentration of SeaPrep® Agarose.

Cell Biology Applications

- Soft agarose cloning of hybridomas[1] and human tumor cells[2,3].
- General cell culture[4]; plant protoplast culture[5].
- Embedding/encapsulation of cells for electron microscopy[6], neuron activity studies[7], transplantation research in rats[8].
- Electrophoresis of cells in a density gradient with reversible gel[9].

Agarose Preparation

In some cases, such as hybridoma cloning when using SeaPrep® Agarose it is advisable to sample different lots of agarose for the desirable gel strength qualities.

When preparing agarose for cell culture work, it is always best to prepare the agarose in water suitable for cell culture and separate from any growth media or nutrients. Agarose solution and media solutions should be prepared at 2X concentrations (i.e., if desired final agarose concentration is 0.6%, prepare a 1.2% agarose solution), autoclaved separately, and aliquoted into useable aliquots.

General Instructions Dissolving

1. Measure an appropriate volume of water or buffer (room temperature) into a beaker or wide mouth flask that is 2-4 times the volume of the solution to be heated.
2. Slowly sprinkle a weighed amount of SeaPrep® agarose powder into the water or buffer with rapid stirring such as with a magnetic stir bar.
3. Weigh the vessel and its contents, then cover it with vented plastic wrap.
4. Heat to boiling with continuous rapid stirring. SeaPrep® agarose sometimes lumps, so gentle boiling with stirring may be required for 20-30 minutes.
5. Remove the plastic wrap, weigh the vessel and contents and correct for evaporation.
6. Cool the solution. SeaPrep® agarose solutions can be poured at room temperature. Poured gels should be cooled **below** their gelling temperature (refrigerated) for at least two hours to achieve maximum gel strength.

Microwave Dissolving

Instructions and timing are based on a single 250 ml flask containing 100 mL of solution in a 720-watt oven. Adjustments should be made for different solution volumes and/or multiple solutions in the microwave oven.

1. Disperse agarose as usual and weigh container.
2. REMOVE MAGNETIC STIR BAR and heat the covered vessel in the microwave oven at MEDIUM (70%) power setting for 2 minutes.
3. GENTLY swirl to resuspend any settled powder and gel pieces.
4. Reheat at HIGH (100%) for 1-2 minutes until the solution comes to a boil.
5. GENTLY swirl to thoroughly mix contents.
6. Remove plastic wrap and adjust for evaporation.

Microwave Melting of Gels

1. Loosen cap of container.
2. Set container in microwave oven and heat at DEFROST (50%) power setting for 1 minute (This is for a 100-mL gel volume; **use shorter times for smaller volumes**.)

3. GENTLY swirl contents to break up gel matrix. If necessary, reheat at DEFROST briefly until all gel pieces are melted.
4. Cool to an appropriate temperature.

Procedure for Autoclaving Agarose

1. Choose a flask that is 2-4 times the volume of the solution.
2. Add water to the flask.
3. Sprinkle in the pre-measured agarose powder at a 2X final agarose concentration.
4. Cover the flask with aluminum foil.
5. Place the flask in the autoclave.
6. Sterilize the agarose by autoclaving for 5 minutes at 15 lb/in^2.
   Note: Agarose may lose gel strength when exposed to longer periods of time in the autoclave.
7. Once the agarose solution has cooled, aliquot into useable aliquots and store at 4ºC prior to use.

General Procedure for Using Agarose in Culture Medium

1. Remelt the agarose by placing in a hot water bath or microwave.
2. Allow the agarose solution to cool to 37ºC.
3. Prewarm the 2X media solution to 37ºC.
4. Mix equal volumes of the sterile 2X agarose solution with sterile 2X media containing growth factors and nutrients.
5. Cast the agarose/media solution into plates or sterile culture tubes.
6. Allow the agarose solution to gel for 20 minutes if using as a feeder or overlay or maintain the solution at 37ºC if using as a liquid culture.

References

1. Civin, C.I; and Banquerigo, M.L. **Journal of Immunological Methods** 1983, **61**, 1-8.
2. Pavlik, E.J.; et al. **InVitro** 1983, **19**,538-550.
3. Bagasra, O.; et al. **Cancer Immunology and Immunotherapy** 1985, **20**, 55-60.
4. Liebow, C.; et al. **Digestive Diseases and Sciences** 1983, **28**, 940.
5. Adams, T.L; and Townsend, J.A. **Plant Cell Reports** 1983,**2**,165-168.
6. Stausbauch, P.;et al. **Journal of Electron Microscopy Techniques** 1985,**2**,261-262.
7. Mooney, R.; and Waziri, R.; **Journal of Neuroscience Methods** 1982,**5,**249-251.
8. Howell, S.L.; et al. **Journal of Physiology** 1982, **324**, 20P.
9. Plank, L.D.; et al. presentation at “Electrophoresis 84”, Univ. of Arizona, Tucson, AZ, 1984.

Order Information:

Catalog No.	Size
50302	25g

For more information contact Technical Service at (800) 521-0390 or visit our website at **www.Lonza.com**

Related Products:
Lonza Cells and Media

For Research Use Only. Not for use in diagnostic procedures.