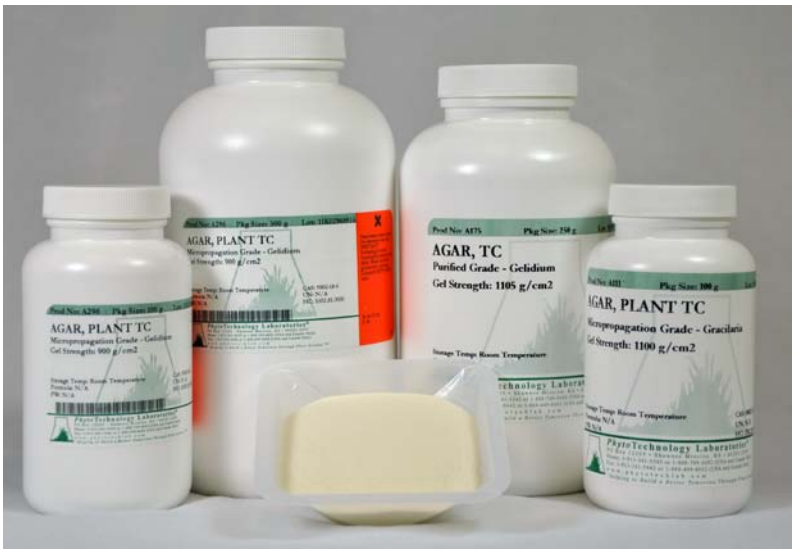
Agars & Other Gelling Agents

PhytoTechnology Laboratories® offers a variety of agars and other products that can be used as gelling agents. These products have been tested in plant tissue culture applications to ensure the highest quality.



Agar is a natural product, which is produced from a family of red seaweeds (*Rhodophycae)* primarily from two genera, *Gellidium and Gracillaria.* Agars produced from *Gelidium* typically have higher gel strength than those from *Gracilaria*. *Geledium* is a small, slow growing plant. Efforts to cultivate it in tanks or ponds have been biologically successful; however, it has generally proved to be uneconomic.

Gracilaria was once considered unsuitable for agar production because the gel strength was too low. However, in the 1950's it was discovered that pre-treatment of the seaweed with alkali before extraction lowered the yield but gave an agar with higher gel strength.

Agar has long been used to solidify media for plant tissue culture. The type of agar or gelling agent used can influence the growth of the tissue in culture. Both purity and cost of the gelling agent are important factors in any research or production operation. PhytoTechnology Laboratories® has expanded its line of gelling agents to allow greater selection in choosing the plant cell culture tested gelling agent for your particular requirement.

Since agar is derived from a biological source, the properties of this product can vary from lot to lot. PhytoTechnology Laboratories® screens every lot of agar prior to accepting it for use in plant tissue culture. We evaluate each lot for clarity, gel strength, biological growth of plants along with other physiochemical properties of the product. We suggest for critical research or production criteria, you may want to screen each lot prior to purchase. PhytoTechnology Laboratories® will reserve material for your use for a period of up to six months.

***Phyto*Technology Laboratories®**
P.O. Box 12205; Shawnee Mission, KS 66282-2205
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Web Site: www.phytotechlab.com

Technical Information

Listed in the following table are some criteria, which may help you select the appropriate product for your application.

Agar and Other Gelling Agents Selection Guide

Product Number	Product Description	Use	Powder Color	Gel Color	Gel Strength	Recommended Concentration
A111	Agar, Micropropagation Grade	General Plant TC, Micropropagation	White to Off-White	Opaque to Off-White	Minimum 900 g/cm^2	5.0 to 8.0 g/L
A296	Agar, Bacteriological Grade	General Plant TC, Microbiology Research	Tan	Tan to Straw Colored	Minimum 700 g/cm^2	6.0 to 12.0 g/L
A175	Agar, Purified	High purity agar for embryogenic or other critical research	White	Opaque to Off-White	Minimum 700 g/cm^2	8.0 to 10.0 g/L
A133	AgarGellan—A proprietary blend of agar and Gellan Gum	General Plant TC/ Micropropagation	Off-White	Opaque to Off-White	TBD	3.5 to 5.0 g/L
A105	Agarose, Low Gelling Temp. (26-29° C)	Plant TC Research	White to Off-White	Opaque to Off-White	Minimum 250 g/cm^2	6 to 10 g/L
A110	Agarose, Low EEO (>35° C)	Plant TC Research	White to Off-White	Opaque to Off-White	Minimum 800 g/cm^2	6 to 10 g/L
A1315	Agarose, Low Gelling Temp. SeaPlaque®	Plant TC Research	White to Off-White	Colorless	TBD	6 to 10 g/L
A108	Alginic Acid	Embryo, Cell, and Protoplast Encapsulation	White to Off-White	Opaque to Off-White	TBD	1.75 -4.0% (w/v)
C257	Carrageenan – Gelcarin GP 812®	General Plant TC/ Micropropagation	Tan	Tan	TBD	8 to 10 g/L
C2000	Carrageenan, High Clarity	General Plant TC/ Micropropagation	Tan	Slight Yellow Tint	Minimum 800 g/cm^2	8 to 10 g/L
G434	Gellan Gum	General Plant TC /Micropropagation	White to Off-White	Colorless	Minimum 800 g/cm^2	1.5 to 2.5 g/L
G3251	Gelzan™ – Trademarked product of CP Kelco®	General Plant TC/ Micropropagation	White to Off-White	Colorless	Minimum 400 g/cm^2	1.5 to 2.5 g/L

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Preparation of Agar (Product No. A111, A175 and A296)

Agar is by far the most common gelling agent used in plant tissue culture. It is used at a wide range of concentrations from 6 g/L for a very solid gel to 10 g/L for a brick-like gel; however, 6-8 g/L is the more commonly used range. Agar should be added slowly to the media while stirring or agitating. The pH of the media should be adjusted after the inclusion of agar. This is critical for proper gelling since the pH of the agar can vary from lot to lot. The agar gel generally becomes softer the more acidic the pH of the medium. Many types of agar will not properly gel at a pH of less than approximately 5.2.

Preparation of Agargellan™ (Product No. A133)

Agargellan is a proprietary blend of micropropagation grade agar and Biotech grade CultureGel (Gellan Gum) that was developed to help control hyperhydricity in plant tissue cultures. Agargellan provides the positive attributes of both agar and Gellan Gum and is superior to Gellan Gum alone in applications where hyperhydricity is a problem. It also serves as an economical alternative to agar for many species. Agargellan produces a semi-clear gel which allows for improved detection of contamination, relative to agar gels. Agargellan should be used at a concentration of 3.5 - 5.0 g/L. As with agar and Gellan Gum, is should be added to stirring medium that is room temperature.

Preparation of Alginate Gel/Beads (Product No. A108)

Alginic Acid has been used for a number of cell and tissue culture applications including use as a physical support similar to agarose and for the preparation of gelled beads. Both of these applications have been used to immobilize and embed suspension cells and protoplasts (Adaoha Mbanaso and Roscoe, 1982; Chee and Cantliffe, 1989; Draget *et al.*, 1988; Larkin *et al.*, 1988). Alginate solutions form a reversible gel at room temperature in the presence of calcium ions. The gel can be re-liquified with a chelating agent, such as citrate. Cells imbedded in gel matrices can be manipulated with significantly less physical damage during handling than cells in liquid medium.

Alginate should be dissolved in a low calcium (e.g., 2 mM) buffered medium at 1.75 - 4.0% (w/v). If protoplasts are to be embedded in the gel then the medium should contain an appropriate osmoticum. Alginic acid will require several hours to dissolve. As it dissolves, the solution will increase in viscosity. This viscosity will negate filter sterilization through a 0.2 µm membrane; a 0.45 µm membrane can be used. While some researches have indicated that alginate solutions can be autoclaved, Larkin et al. (1988) noted a reduction in bead-making capacity with increased autoclave time.

If protoplasts are to be embedded, they should first be concentrated by centrifugation in a low calcium medium and added to alginate at an appropriate density (e.g., 1 x 10^5 cells/ml). The protoplast-alginate solution is added drop-wise to a solution containing 50 mM $CaCl_2$ and an appropriate osmoticum. Each droplet will for a bead. The beads should remain in the $CaCl_2$ for up to 45 minutes to ensure optimum gel matrix formation.

Preparation of CultureGel™ Gellan Gums (Product No. G434 and G3251)

CultureGel Gellan Gum is an alternative gelling agent to agars. Gellan Gum is produced from a bacterial substrate composed of glucuronic acid, rhamnose, and glucose. G434 is a biotech grade that is very clear that produces high strength gel that is

significantly clearer than agar gels. This aids in the detection of microbial contamination. Gellan gum offers an economical alternative to agar in many *in vitro* applications. Gellan gum will form a gel in the presence of mono- or divalent cations; the latter being more efficient, e.g., calcium, magnesium; however, gellan gum is not recommended for use with DKW (9.3 mM Ca++) or other media e.g., Quoirin & Lepoivre Basal Salt Mixture containing high calcium levels as they have shown to produce a soft and cloudy gel.

Gellan gum is typically used at a concentration from 2 to 4 g/L. It is suspended in medium that is room temperature or colder. Attempting to suspend it in hot medium will usually result in an incomplete, lumpy suspension that will not melt and dissolve uniformly when autoclaved. It should be added to medium after all heat-stable supplements have been added.

Preparation of Carrageenan (Product No. C257 and C2000)

Carrageenan is produced from a family of red seaweeds, *Rhodophyceae*, of many different genera such as *Chondrus, Eucheuma, Gigartina, and Iridaea*. These different genera produce different types of carrageenans such as kappa, lambda, and iota. Product No. C257 is Gelcarin GP 812® which is a registered trademark of FMC BioPolymer. It is a kappa-type carrageenan that forms a strong, rigid gel in the presence of potassium ions often under the process called potassium precipitation.

When carrageenan is dissolved properly, it will produce a rigid gel. Carrageenan is typically used at a wide range of concentrations from 6 g/L to 10 g/L. It is suspended in a medium that is at room temperature or colder like agar. Carrageenan should be added last since the medium will become viscous, as carrageenan is a water-soluble polymer; the viscosity of carrageenan increases with concentration and decreases with temperature. Moreover, carrageenan should also be added slowly to an agitated medium to help prevent clumping of the carrageenan and to create a uniform suspension. A lumpy suspension of carrageenan will not dissolve uniformly when autoclaved. Next, the pH of the medium should be adjusted. After autoclaving, stir the medium to distribute the melted carrageenan uniformly into the solution.