Instructions for use

GELRITE®

0039

Gellan Gum for Microbiological Applications
GELRITE® is a trademark of Merck & Co., Inc. (Rahway, NJ) Kelco Division, U.S.A.

Developed especially for use as a gelling agent for microbiological media, GELRITE® gellan gum is a highly-purified, natural anionic heteropolysaccharide that forms rigid, brittle, agar-like gels at approximately half the use level of agar, in the presence of soluble salts.[1,2,3]
GELRITE® is the ideal gelling agent for a wide range of gelling applications, for the following reasons:

Advantages of GELRITE® Compared to Agar

- GELRITE® gellan gum may be used at approximately half the use level of agar
- GELRITE® , produced by a tightly-controlled fermentation process, has consistent product quality. GELRITE® is unaffected by the vagaries of natural conditions which affect the basic properties of agar.
- GELRITE® gels are remarkably clear in comparison to those formed with agar.
- Gels prepared with GELRITE® set faster than those made with agar. In microbiological applications this reduces plate preparation time.
- Gels prepared with GELRITE® are stable at high temperatures. In microbiological media, this supports incubation required by thermophilic microorganisms.
- GELRITE® contains no contaminating matters (e.g., phenolic compounds) as those found in agar that are toxic to certain sensitive organisms.

Ease of Processing with GELRITE® Media

- GELRITE® gellan gum disperses and hydrates easily in either hot or cold deionized water, forming viscous solutions in cold distilled water.
- In the presence of soluble salts, GELRITE® can be used to provide high gel strength at low GELRITE® concentrations (normally at approximately half the concentration required for agar).
- At high temperatures, the low viscosity of GELRITE® solutions facilitates pipetting, pumping, and pouring upon cooling, GELRITE® solutions gel quickly and uniformly.
- GELRITE® is able to withstand normal autoclaving conditions.
- GELRITE® is generally resistant to enzymatic degradation.
- GELRITE®itself is chemically inert to most biological growth media additives (additive must be heated to just above GELRITE® gel point before incorporation).

[1] For further explanations of GELRITIE`gellan gum as an agar replacer, refer to Kelco Applications Bulletin CD-33.
[2] U.S. patents 4, 326, 052 and 4, 326, 053.
[3] GELRITE® gellan gum may be marketed as an in vitro diagnostic device (U.S. Food & Drug Administration review under 21 U.S.C. 510(k)).

Quality of Microbiological Media Prepared with GELRITE®

- GELRITE® gellan gum gels have proven to be a suitable growth matrix for a wide variety of microorganisms, including those traditionally cultured on agar plates as well as other species not easily grown on other substances.
- GELRITE® gels are exceptionally clear, making them an excellent analytical tool.
- GELRITE® gels have essentially the same shelf life as agar gels.

Chemical and Physical Properties

Chemical composition: polysaccharide comprising glucuronic acid, rhamnose and glucose.
Physical state: dry powder
Solids: 85-95%
Color: white

Media can be formulated with 0.6-0.8 % GELRITE® gellan gum and 0.10 % $MgSO_4*7H_2O$ to achieve gels strengths ranging from 225-500 g/cm² (Marine Colloid gel tester).
GELRITE® gellan gum is a linear polysaccharide comprising glucuronic acid, glucose, rhamnose, and O-acetyl moieties. Recent research suggests that the GELRITE® gellan gum tetrasaccharide repeating unit has the structure: [1] →3)ß-D-Glcp-(1→4)-ß-D-GlcpA-(1→4)-ß-D-Glcp-(1→4)-α-L-Rhap-(1→

Characteristics of GELRITE® Gels

Gel structure is integral to choosing suitable cultural media that are used to isolate pure bacterial cultures, to characterize colonial morphology, to perform microbiological tests, and to enumerate microbes. Traditionally, agar has been used as the matrix for solid media. In the presence of soluble salts, GELRITE®gellan gum has a gel structure that effectively supports microbial growth (refer to bulletin CD-27) and is viewed as an excellent alternative to agar as a growth medium.
Gels prepared by autoclaving at 121 °C and 15 psi for 15 minutes. Gel strength determined using a Marine Colloids Gel Tester with small plunger at slow speed.

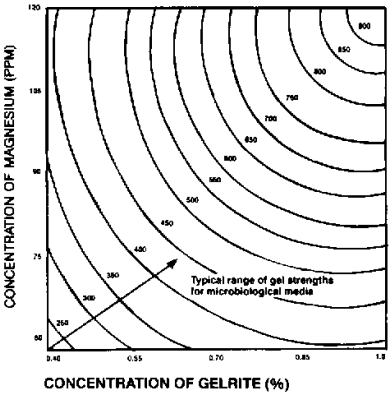


Figure 1. Response surface curve for gels showing gel strength as a function of GELRITE® gellan gum and magnesium concentrations.

Gel Strength-Gel strength is a useful parameter in determining appropriate gel structure for support of microbial growth. The gel strength of GELRITE® gels is highly dependent on the type of salt added, the GELRITE® concentration (as shown in Figure 1), and the soluble salt concentration (as shown in Figures 2 and 3). Figure 1 is a response surface methodology (RS) curve which emphasizes the GELRITE® and $MgSO_4*7H_2O$ relationship that results in varied gel strengths. It should be noted that a gel strength of 250-450 g/cm² is a commonly found gel strength range of agar in microbiological media. The appropriate GELRITE® and $MgSO_4*7H_2O$ concentrations that yield this gel strength may be estimated from the RS curve. It should be noted that frequently a nutrient medium containing simple salts does not require additional salt to form an effective gel, e.g., Brain Heart Infusion.
Magnesium is the preferred ion in microbiological media applications. It produces in most cases a thermally reversible gel.

[1] O'NEILL, M. A. and others. "Structure of the acidic extracellular gelling polysaccharide produced by Pseudomonas elodea." *Carbohydrate Research, vol. 124,* no. 1 *(1983) 123-133.*
JANNSON, RE. and others. "Structural studies of gellan gum, an extracellular polysaccharide eleborated by Pseudomonas elodea." *Carbohydrate Research, vol. 124,* no. 1 *(1983) 135-139.*

Effect of Salts

GELRITE® gellan gum requires the presence of either monovalent or divalent cations for gelation.
GELRITE® gellan gum shows unique versatility in its gel **characteristics:** Gels can be modified as desired by changing the concentration and type of cation in the GELRITE® media.
As shown in Figures 2 and 3, divalent cations such as magnesium or calcium have a much more profound effect on gel strength than do monovalent ions such as sodium or potassium. Of the cations tested, calcium exerts the most profound effect on the gel strength of GELRITE® . In fact, gels prepared with calcium do not remelt under normal sterilization conditions.
Gelled media prepared using GELRITE® and a small amount of CaCI, (0.1 %) are remarkably stable at 80 °C for at least 10 days. There is no syneresis in this gel system, whereas the agar gel and the $MgSO_4$-mediated GELRITE® gel show severe syneresis under these conditions. This feature is especially suitable to the culturing of many thermophiles.[1]

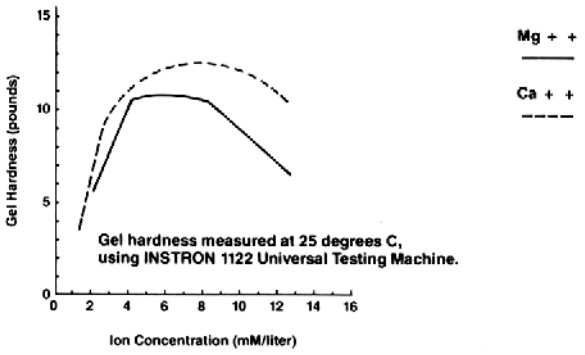


*Figure 2.
Effect of Divalent Salts on Gel Hardness 1 % GELRITE Gels

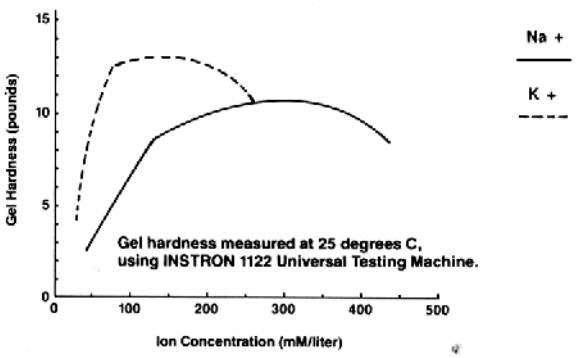


*Figure 3.
Effect of Monovalent Salts on Gel Hardness 1 % GELRITE Gels

GELRITE® requires both a heating cycle and the presence of cations for gelation to occur GELRITE® requires heating to **approximately 100 °C** to achieve complete solubility in the presence of ions. The GELRITE® solution will then remain essentially non-viscous until, upon cooling, it reaches its gel setting point, at which time gelation occurs very rapidly, much more rapidly than agar for example. The gel setting temperature is a function of the GELRITE® and cation concentrations and can vary from 35 to >5O °C at 1 % GELRITE® concentration, as illustrated in Figures 4 and 5. The gel setting temperature will sometimes increase
10-15 °C if the GELRITE® solutions are allowed to stand in a water bath for more than 30-40 minutes. The GELRITE® media can be kept fluid for longer periods of time by increasing the temperature of the water bath by 15 °C above the stated gel set temperature for a particular medium.
Note that for all cations used, gel setting temperature increased with increasing cation concentration, even though gel hardness increases to a maximum then decreases.

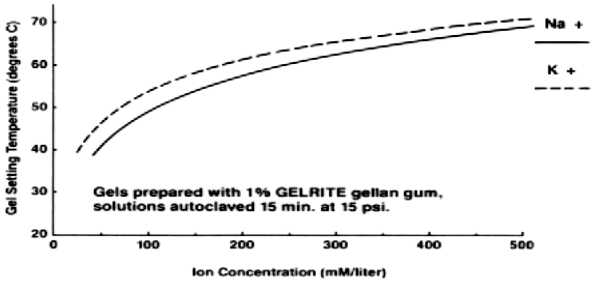


*Figure 4.
Effect of Monovalent Salts on Gel Set Point 1 % GELRITE Gels

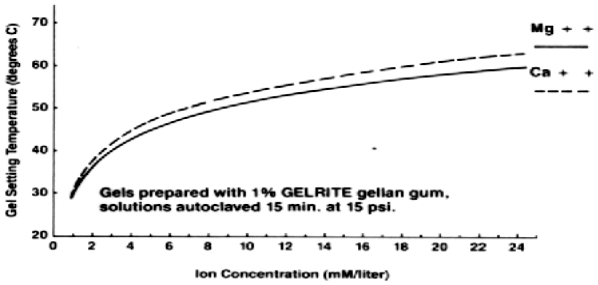


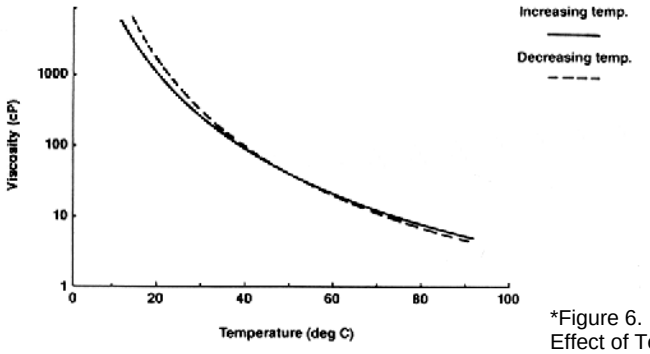
*Figure 5.
Effect of Divalent Salts on Gel Set Point 1 % GELRITE Gels

* Figures 2-5:
Gels were prepared with a 1 % GELRITE® gellan gum solution using varying amounts of NaCI, KCI, $CaCl_2*2H_2O$ or $MgCl_2*6H_2O$ Solutions were autoclaved 15 minutes at 15 psi. Gel hardness was measured at 25 °C using the INSTRON 1122 Universal Testing Machine.

[1] For information on GELRITE® as a gelling agent in media for thermophilic microorganisms, see: Lin, CC and L.E. Casida, Jr. Applied *and* Environmental *Microbiology, vol*. 47, no. 2 *(Feb. 1984*) pp. 427-429.

Effect of Temperature

As illustrated in Figure 6, GELRITE® gellan gum solutions demonstrate thermally reversible viscosity changes. The viscosity decreases sharply with increasing temperature, but returns to its original value upon cooling. When prepared in ion-free water, GELRITE® solutions may be heated and cooled without gelation occurring.[1]



GELRITE® has good thermal stability and is able to withstand a normal autoclaving cycle (121 °C, 15 psi, 15 minutes) without losing significant gel strength. When compared to agar, its thermal stability is remarkably similar during the initial autoclaving cycle, which is most significant since most solid media undergo only one such sterilization cycle.

*Figure 6.
Effect of Temperature on Viscosity 1 % GELRITE Solution

Compatibility with Nutrient Additives

GELRITE® gellan gum is completely compatible with nutrient additives commonly used with agar gels. GELRITE® remains inert to most additives with the exception of soluble salts (see Figures 1 and 2).

Optical Clarity

GELRITE® gellan gum gels are optically clear as noted in Table 1. As demonstrated by the percentage transmittance, GELRITE® gels are optically as clear as or clearer than compatible agar gels.

Table 1. Light Transmittance of Gelled Media Prepared with Agar and with GELRITE® gellan gum $MgCl_2*6H_2O$

Medium	Agar (%)	GELRITE® (%)	Wavelength (nm)	% Transmittance*
Difco Nutrient Broth	1.5	-	530	52.5
Difco Nutrient Broth	-	0.8	530	73.0
Brain Heart Infusion	1.5	-	530	42.5
Brain Heart Infusion	-	0.75	530	55.7
Trypicase Soy Broth	1.5	-	530	54.9
Trypicase Soy Broth	-	0.75	530	73.2
Potato Dextrose Broth	1.5	-	535	63.0
Potato Dextrose Broth	-	0.75	535	66.5

* % transmittance was measured on a Spectronic 20. The blank was broth control for each medium. All media were autoclaved for is minutes at 121 °C and 15 psi. % transmittance was measured after gelation. The wavelengths selected gave lowest transmittance for each medium.

Conformation

Preliminary X-ray fiber diffraction studies[2] of gellan gum suggests that a three-fold helical conformation exists in the solid state. The O-acetyl substituents on gellan gum appear to affect the packing of these helices into crystalline domains. The most crystallinity is seen in the absence of O-acetyl groups; this may relate to the formation of rigid, brittle gels from solution.

[1] Viscosity of a 1 % GELRITE® solution in deionized water, measured at 60 rpm on a Brookfield LVT viscometer using the appropriate spindle.

[2] CARROLL, W; M.J. MILES; and V.J. MORRIS (ARC Food Research Inst., Norwich) "Fibre-diffraction studies on the extracellular polysaccharide from Pseudomonas elodea." International Journal of Biological Macromolecules, Vol. 4 (Dec. 1982) pp. 432-433.

Synergism with Other Polymers

For industrial gelling applications other than those in the microbiological media area, GELRITE® gellan gum exhibits a useful synergism with other polymers.
Synergism studies show evidence of gel strength enhancement when GELRITE® is blended with gelatin or gum arabic. At 0.5 % total gum concentrations in standard tap water[1], 1:1 blends of GELRITE® and Gelatin or gum arabic show respectively an approximate 60 % and 40 % increase in gel strength relative to 0.5 % GELRITE® alone.

Storage and Handling

Storage and handling procedures should follow the normal practices recognized as desirable for naturally derived polymeric gelling agents. The gel strength of the dry powder is retained, even after prolonged storage at 50 °C (122 °F) and will recover from freezing. However, as with any polysaccharide, such changes in temperature may tend to reduce its stability and should be avoided. GELRITE® gellan gum should be stored tightly closed, in a cool (<60° F) dry place for maximum shelf life.

Toxicity

GELRITE®gellan gum caused no oral toxicity in rats fed the pure product at a single dose of 5000 mg/kg. From the results of eye irritation tests, GELRITE® is not considered to be an eye irritant. During dust inhalation tests, no toxic symptoms were exhibited by rats exposed to concentrations averaging 6.09 mg/liter for a 4-hour period. The lungs of these necropsied rats appeared normal.
Mutagenicity tests show GELRITE® to be negative in the Ames test. No special precautions are required to handle GELRITE® gellan gum. Other studies on the safety of gellan gum are in progress. Please contact your Kelco technical service representatives should you require further information.

Gellan Gum Thermal-Reversible Gelling Agent as an Agar Replacer

The gels formed by GELRITE® gellan gum in the presence of soluble salts have been tested and found to be a suitable growth matrix for most clinical and nonclinical organisms. The gel characteristics necessary to function as a matrix for solid media include thermal-reversibility[2], optical clarity, and compatibility with nutrient additives. (Refer to Bulletin CD-32 for further product descriptions.)
Some benefits of using GELRITE® are:

- wet enough to support growth
  but
- dry enough to separate colonies
  AND
- fine enough to prevent migration of organisms within the gel
  but
- coarse enough to allow diffusion of macromolecular nutrients.

[1] Standard tap water is formulated to represent a typical water supply containing 1,000 ppm sodium chloride and 147 ppm calcium chloride dihydrate.
[2] Under certain conditions gels can be made

GELRITE® Comparison with Agar

Traditionally, agar has been used as a matrix for solid media suitable for isolating pure cultures. The agar used has been clear and smooth in texture so that characterization of colonial morphology by shapes, textures, pigmentations, etc., could be readily apparent. All tests using GELRITE® as the gelling component for nutrient media have shown that GELRITE® rapidly sets into a smooth gel that is as clear as or clearer than agar, thus facilitating characterization of organisms.[1-2]

The gel strength is dependent on the type of soluble salt added, the GELRITE® concentration, and the salt concentration. (Refer to Bulletin CD-32 for graphic and tabular data.) Some GELRITE® salt combinations appear to be more suitable growth matrices for particular microorganisms. GELRITE® gels with $MgSO_4*7H_2O$ are extremely effective. Gels made with a 0.75 % concentration of GELRITE® and a 0.1 % concentration of $MgSO_4*7H_20$ are comparable to gels made with 1.5 % agar. These gels range from approximately 225-500 g/cm^2 as measured on an Instron 1122.

Preparation of GELRITE® Nutrient Broth Gels

Procedure
Method 1 (Preferred)

1. To make 1 liter of nutrient broth containing GELRITE® as the solidifying agent, add and dissolve the following ingredients to 1000 ml distilled water in the order listed:
   8.0 g Nutrient Broth (dehydrated powder)
   1.0 g $MgSO_4*7H_2O$
   Then add 8.0 g GELRITE® with vigorous stirring to disperse.
2. Heat to boiling while stirring to dissolve the GELRITE®.
3. Autoclave the above mixture for 15 minutes at 121 °C, 15 psi.
4. Gently swirl the mixture for 15 seconds. **(CAUTION** should be taken when handling a superheated mixture to avoid burns.)
5. Cool to 60 °C
6. Pour into petri dishes. (Note that GELRITE® gels set rapidly, thus allowing more plates to be poured in a shorter period of time.)

Method 2

1. Using same amounts of components as in METHOD 1, add GELRITE® to distilled water.
2. Bring mixture to boil.
3. Add nutrient broth and magnesium salt.
4. Autoclave under conditions in METHOD 1 and continue from step 4 in METHOD 1.

The resulting gel strength obtained from nutrient broth, using 0.8 % w/w GELRITE® as the gelling agent, is 700 ±150 g/cm^2, which is comparable to the same system gell with ~1.5 % agar. The gel setting point of the GELRITE® system is 41 °C ± 2 °C.

[1] The U.S. Food & Drug Administration has determined under Section 510(k), that GELRITIE gellan gum is substantially equivalent to agar.
[2] Gellan gum is covered by U.S. patents non-thermal-reversible. US-4326052 and US-4326053.

Preparation of GELRITE® Eosin Methylene Blue (EMS) Gels

Procedure
Method 1 (Preferred)

1. To make 1 liter of Eosin Methylene Blue (EMB) containing GELRITE® as the solidifying agent, add and dissolve the following ingredients to 1000 ml distilled water in the order listed:
   22.5 g EMB (dehydrated powder, without agar)
   1.0 g $MgSO_4*7H_2O$
   Then add 8.0 g GELRITE® with vigorous stirring to disperse.
2. Heat to boiling while stirring to dissolve the GELRITE®.
3. Autoclave the mixture for 15 minutes at 121 °C, 15 psi.
4. Gently swirl the mixture for 15 seconds. (CAUTION should be taken when handling a superheated mixture to avoid burns.)
5. Cool to 60 °C.
6. Pour into petri dishes. (Note that GELRITE® gels set rapidly, thus allowing more plates to be poured in a shorter period of time.)

Method 2

1. Using same amounts of components as in METHOD 1, add GELRITE® to distilled water.
2. Bring mixture to boil.
3. Add EMB and magnesium salt.
4. Autoclave under conditions in METHOD 1 and continue from step 4 in METHOD 1.

The resulting gel strength obtained from EMB, using GELRITE® as the gelling agent, is 475 ± 75 g/cm^2, which is comparable to the same system gelled with ~1.5 % agar. The gel setting point of the GELRITE® system is 42 °C ±2 °C.

Preparation of GELRITE® Salmonella Shigella (SS) Gels

Procedure
1. To make 1 liter of Salmonella Shigella (SS) media, containing GELRITE® as the gelling agent, dissolve 46.5 g SS media (Dehydrated powder without agar) in 1,000 ml distilled water. Then add 6.0 g GELRITE® with vigorous stirring to disperse.
2. Heat with repeated stirring and boil for one minute.
3. Cool to 60 °C.
4. Pour into petri dishes. (Note that GELRITE® gels set rapidly, thus allowing more plates to be poured in a shorter period of time.)

The resulting gel strength obtained from SS, using GELRITE® as the gelling agent, is 425 ±110 g/cm^2, which is comparable to the same system gelled with 1.5 % agar. The gel setting point of the GELRITE® system is 53 °C ±2 °C.

Preparation of GELRITE® Tryptic Soy Gels

Procedure
Method 1 (Preferred)

1. 1.To make 1 liter of Tryptic Soy Media containing GELRITE® as the solidifying agent, add and dissolve the following ingredients to 1000 ml distilled water in the order listed:
   27.0 g Tryptic Soy Media (Dehydrated powder without agar)
   1.0 g $MgSO_4*7H_2O$
   Then add 8.0 g GELRITE® with vigorous stirring to disperse.
2. Heat to boiling with stirring to dissolve the GELRITE®.
3. Autoclave the above mixture for 15 minutes at 121 °C, 15 psi.
4. Gently swirl the mixture for 15 seconds. (CAUTION should be taken when handling a superheated mixture to avoid burns.)
5. Cool to 60 °C (except for blood plates, which must be cooled to 50 °C prior to blood addition to avoid hemolysis).
6. Pour into petri dishes. (Note that GELRITE® gels set rapidly, thus allowing more plates to be poured in a shorter period of time.)

Method 2

1. Using same amounts of components as in METHOD 1, add GELRITE® to distilled water.
2. Bring mixture to boil.
3. Add Tryptic Soy medium and magnesium salt.
4. Autoclave under conditions in METHOD 1 and continue from step 4 in METHOD 1.

The resulting gel strength obtained from Tryptic-Soy gels, using GELRITE® as the gelling agent, is 770 ±110 g/cm^2, which is comparable to the same system gelled with ~1.5 % agar. The gel setting point of the GELRITE® system is 52 °C ±2 °C.

Preparation of GELRITE® Tryptic Soy/Blood Gels

The present method for the preparation of blood plates made using GELRITE® involves preparing two solutions and combining them after sterilization. The gel setting point of the GELRITE® -tryptic soy/blood mixture is 48 °C ±1 °C, therefore it is critical to maintain the temperature at 50 °C or above once the solutions are combined. A batch process is outlined below giving the details for the preparation of a 200 ml volume. Because of the critical temperature of the gel setting point, it is difficult to prepare volumes greater than 200 ml using a batch process. However, in a multifeed, continuous system, this difficulty would be circumvented.

Procedure

1. Dissolve 5.4 g tryptic soy medium in 190 ml of distilled water.
2. Divide the solution into two parts. One portion should contain 130 ml volume (solution A) and the other portion should be 60 ml in volume (solution B).
3. Add 1 drop of a 1-10 dilution of POURITE[1] to solution A. To solution B, add 2 gms GELRITE®.
4. Autoclave the above solutions for ~15 minutes at 121 °C, 15 psi.
5. Gently swirl the solutions for 15 seconds. (CAUTION should be taken when handling a superheated mixture to avoid burns.)
6. Cool solution A to 50 °C and aseptically add 10 ml of defibrinated sheep blood which has been warmed to 25 °C. Mix well to insure even distribution of blood.
7. While mixing solution A, immediately add solution B, which has been previously cooled to 70 °C.
8. Pour into petri dishes. (Note that GELRITE® gels set rapidly, thus allowing more plates to be poured in a shorter period of time.)

The resulting gel strength obtained for tryptic soy/blood plates, using GELRITE® as the gelling agent, is 360 ±20 g/cm^2, which is comparable to the same system gelled with ~1.5 % agar.

[1] Trademark of: American Scientific Products, Division of American Hospital Supply Corp.

Preparation of GELRITE® Triple Sugar Iron (TSI) Gels

Procedure

1. To make 1 liter of Triple Sugar Iron (TSI) medium containing GELRITE® as the solidifying agent dissolve 46.5 g TSI medium (Dehydrated powder without agar) in 1,000 ml distilled water. Then add 6.0 g GELRITE® with vigorous stirring to disperse.
2. Heat with repeated stirring and boil for one minute to dissolve completely.
3. Distribute into test tubes and autoclave for 15 minutes at 121 °C, 15 psi.
4. After autoclaving, slant tubes to prepare deep butts.

The resulting gel strength obtained from TSI, using GELRITE® as the gelling agent, is 350 ±25 g/cm^2, which is comparable to the same system gelled with ~1.5 % agar. The gel setting point of the GELRITE® system is 49 °C ±2 °C.

Preparation of Plant Tissue Culture Gels

Procedure

1. To make 1 liter of gelled media combine:
   2 g GELRITE® and all other components[1] in 1,000 ml of distilled water.
2. Heat with repeated stirring and boil for one minute to dissolve completely.
3. Distribute into test tubes and autoclave for 15 minutes at 121 °C, 15 psi.
4. Allow to cool.

Preparation of GELRITE® Chocolate Gels

Procedure:

1. To make one liter of chocolate medium containing GELRITE® as the solidifying agent, first add 25 gms of Heart Infusion broth, and 7.5 gms of GELRITE® to 1000 ml distilled water. Stir the GELRITE® suspension vigorously to disperse any lumps.
2. Autoclave both aliquots at 121 °C, 15 psi for approximately 15 minutes.
3. Place in a water bath at 80 °C.
4. Add 50 ml of defribinated sheep blood to the Heart Infusion GELRITE® and allow to remain at 80 °C for 15 minutes.
5. Dispense into sterile petri plates. The resulting gel strength obtained with Chocolate GELRITE® medium is 360 gm/cm^2 ±30 gm/cm^2, which is comparable to the same system gelled with approximately 1.5 % agar.

Preparation of GELRITE® Egg Yolk Gels

Procedure:

1. To prepare 1 liter of egg yolk medium containing GELRITE® as the solidifying agent, first divide the distilled water into two 500 ml aliquots.
2. To one, add 25 gms of Heart Infusion broth and a magnetic stirring bar. To the other, add 7.5 gms of GELRITE®. Stir the GELRITE® suspension vigorously to disperse any lumps.
3. Autoclave both aliquots at 121 °C, 15 psi for approximately 15 minutes.
4. Cool the Heart Infusion broth to 50 °C and the GELRITE® to 70 °C.
5. Add 5 ml of egg yolk to the Heart Infusion broth with mixing (magnetic stirrer).
6. Add the GELRITE® to this mixture with constant stirring (magnetic stirrer).
7. Dispense into sterile petri plates.

The resulting gel strength obtained with egg yolk medium solidified with GELRITE® is 200 gms/cm^2 which is comparable to the same system gelled with approximately 1.5 % agar.

[1] Should there be any components that cannot be autoclaved, these can be added after autoclaving. Add as liquids while the GELRITE® gellan gum solution is between 55-60 °C.

Preparation of GELRITE® TCBS Gels

Procedure

1. To make 1 liter of TCBS medium containing GELRITE® as the solidifying agent, add and dissolve the following ingredients to 1,000 ml distilled water in the order listed.

Sodium Thiosulfate	10.0 gm
Sodium Citrate	10.0 gm
Oxgall	5.0 gm
Sodium Cholate	3.0 gm
Sucrose	20.0 gm
Pancreatic Digest of Casein, U.S.P.	5.0 gm
Peptic Digest of Animal Tissue, U. S. P.	5.0 gm
Yeast Extract	5.0 gm
Sodium Chloride	10.0 gm
Iron Citrate	1.0 gm
Thymol Blue	0.04 gm
Bromothymol Blue	0.04 gm
Then add 7.5 g GELRITE®	7.5 gm

with vigorous stirring to disperse.

2. Heat with stirring and boil for one minute.
3. Cool to 50-55 °C and pour into plates.

The resulting gel strength obtained with TCBS using GELRITE® as the gelling agent, is 400 ±40 gm/cm^2, which is comparable to the same system gelled with approximately 1.5 % agar.

Evaluation of GELRITE® as an Agar Replacer

Screening tests conducted by Merck Sharp & Dohme Research Laboratories (MSDRL) and by Kelco Research and Development indicate that GELRITE® gels are suitable as agar replacers for performing biochemical and enzymatic tests. The process of enumerating microbes during tests for such things as ecological factors and quality control previously has been limited to agar gels. The GELRITE® gels readily accommodate this process.

An evaluation of the suitability of GELRITE® as an agar replacer for clinical isolates was conducted. Varied nutrient broths compared GELRITE® gels with microbiological-grade agar as the solidifying agent. Approximately 50 clinical organisms with differing growth requirements were tested as indicated in Table I.

Table II identifies nonpathogenic organisms that were tested by Kelco Research and Development laboratories. Once again, test results indicated that GELRITE® gels were comparable or superior to agar in setting, optical clarity, and various gel strengths that could be attained by adjustments of salt and/or GELRITE® concentrations.

The overall performance of the GELRITE® gels was favorably compared to that of the microbiological-grade agar. GELRITE® gels were considered to be superior to agar in their setting properties, clarity, and adjustability of gel strength.

Table I.
Clinical Isolates[6] Culture Media Used in Evaluation of GELRITE® ™ gum vs Agar

Organism		Media
Escherichia coli	CL 1552	Trypticase Soy® (TS), Blood, McConkey (McC), SS, Bismuth, Sulfite, EMB, TSI
Shigella dysenteriae	CL 1726	TS, Blood, McC, SS, Bismuth Sulfite, Brilliant Green, EMB, TSI
Shigella flexneri	CL 1719	TS, Blood, McC, SS, Bismuth Sulfite, Brilliant Green, EMB, TSI
Shigella boydii	CL 1727	TS, Blood, McC, SS, Bismuth Sulfite, Brilliant Green, EMB, TSI
Shigella sonnei	CL 1705	TS, Blood, McC, SS, Bismuth Sulfite, Brilliant Green, EMB, TSI
Edwardsiella tarda	CL 2076	TS, Blood, McC, TSI
Klebsiella pneumoniae	CL 1697	TS, Blood, McC, TSI
Klebsiella ozaenae	CL 904	TS, Blood, McC, TSI
Klebsiella oxytoca	CL 1060	TS, Blood, McC, TSI
Enterobacter cloacae	CL 1779	TS, Blood, McC, EMB, TSI
Enterobacter aerogenes	CL 1548	TS, Blood, McC, EMB, TSI
Enterobacter agglomerans	CL 1387	TS, Blood, McC, EMB, TSI
Hafnia alvei	CL 136	TS, Blood, McC, EMB, TSI
Serratia marcescens	CL 1520	TS, Blood, McC, TSI
Serratia liquefaciens	CL 1977	TS, Blood, McC, TSI
Citrobacter freundii	CL 1663	TS, Blood, McC, TSI
Citrobacter diversus	CL 1519	TS, Blood, McC, TSI
Proteus vulgaris	CL 1190	Blood, McC, TSI, Nutrient (N)
Proteus mirabilis	CL 1772	Blood, McC, TSI, N
Morganella morganii	CL 1555	Blood, McC, TSI, N
Providencia rettgeri	CL 1192	TS, Blood, McC, TSI
Providencia alcalifaciens	CL 787	TS, Blood, McC, TSI
Providencia stuartii	CL 1443	TS, Blood, McC, TSI
Salmonella typhimurium	CL 1866	TS, Blood, McC, SS, Bismuth Sulfite, Brilliant Green, EMB, TSI
Salmonella enteritidis	CL 1362	TS, Blood, Mcc, SS, Bismuth Sulfite, Brilliant Green, EMB, TSI
Salmonella typhi	CL 1935	TS, Blood, SS, Bismuth Sulfite, Brilliant Green, EMB, TSI
Arizona hinshawii	CL 2075	TS, Blood, McC, TSI
Yersinia enterocolitica	CL 1626	TS, Blood, McC, SS, Bismuth sulfite, Brilliant Green, EMB, TSI
Acinetobacter calcoaceticus	CL 1658	TS, Blood, McC, TSI
Haemophilus influenzae	CL 1826	TS, Chocolate
Haemophilus parainfluenzae	CL 1824	TS, Chocolate
Pseudomonas aeruginosa	CL 1560	TS, Blood, McC, TSI
Pseudomonas fluorescens	CL 1542	TS, Blood, McC, TSI
Pseudomonas maitophilia	CL 2016	TS, Blood, McC, TSI
Staphylococcus aureus	CL 1500	TS, Blood, McC, Egg Yolk(EY), Phenyl Ethyl Alcohol(PEA)
Staphylococcus epidermidis	CL 1803	TS, Blood, McC, TSI, EY, PEA
Staphylococcus saprophyticus	CL 1941	TS, Blood, McC, TSI, EY, PEA
Streptococcus pyogenes	CL 1925	TS, Blood
Streptococcus agalactiae	CL 1342	TS, Blood
Streptococcus faecalis	CL 1776	TS, Blood
Streptococcus pneumoniae	CL 1842	Blood, BHI
Streptococcus Group C	CL 909	TS, Blood
Streptococcus Group G	CL 1929	TS, Blood
Flavobacterium odoratum	CL 1435	TS, Blood, McC, TSI
Moraxella species	CL 387	TS, Blood, McC, TSI
Campylobacter fetus subsp. jejuni	CL 2320	TS, Blood
Vibrio cholerae	CL 2020	TS, Blood, EY, PEA
Clostridium perfringens	CL A144	BHI, Blood, EY, PEA
Peptostreptococcus anaerobius	CL A107	BHI, Blood, EY, PEA
Bacteroides fragilis	CL A1 77	BHI, Blood

[6]All isolates are alphanurnerically identified by MSDRL classifications

Table II.
Nonpathogenic Isolates and Culture Media Used in Evaluation of GELRITE® gellan gum vs Agar

Organism		Media
Agromyces ramosus	ATCC 25173	Trypticase GELRITE®
Arthrobacter globiformis	ATCC 8010	Trypticase Soy GELRITE®
Aureobasidium pullulans	NRRL Y-3861	Potato Dextrose GELRITE®
Azotobacter vinelandii	ATCC 9047	Burk's GELRITE®
Beijerinckia lacticogenes	ATCC 19361	Burk's GELRITE®
Erwinia carotovora	ATCC 8061	Nutrient GELRITE®
Nocardia salmonicolor	ATCC 21243	Nutrient GELRITE®
Trichoderma longibrachiatum	ATCC 13631	Potato Dextrose GELRITE®
Zoogloea ramigera	ATCC 25935	Trypticase Soy GELRITE®

All plates were made according to procedures identified in this application bulletin.

Technical Service

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