

NUTRIENT GELATIN (7471)

Intended Use

Nutrient Gelatin is used for the differentiation of microorganisms on the basis of gelatinase production.

Product Summary and Explanation

Gelatin was the first gelling agent used to solidify culture media. The advantages of solid media over liquid media include isolation of pure cultures and the ability to perform plate counts. The disadvantages of gelatin include incubation at 20°C, a temperature that is lower than optimum for growing many microorganisms, and the fact that many organisms metabolize (liquefy) gelatin. Agar later replaced gelatin as a solidifying agent.

Identifying fermentative and non-fermentative Gram-negative bacilli include testing for gelatin liquefaction. If the proteolytic enzyme gelatinase is present, gelatin is hydrolyzed and loses its gelling characteristic.¹ Edwards and Ewing include this test in the differentiation scheme for *Enterobacteriaceae*.² Procedures for performing the standard tube method for gelatin liquefaction are available.²⁻⁴

Principles of the Procedure

The nitrogen, carbon, vitamins, and amino acids are provided by Enzymatic Digest of Gelatin and Beef Extract for general growth requirements in Nutrient Gelatin. Gelatin is the substrate for determining if microorganisms elaborate the proteolytic enzyme to hydrolyze (liquefy) gelatin.

Formula / Liter

Enzymatic Digest of Gelatin 5 g
 Beef Extract 3 g
 Gelatin..... 120 g

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. For Laboratory Use.

Directions

1. Dissolve 128 g of the medium in one liter of purified water.
2. Heat with frequent agitation to 50°C to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and very light to light beige.

Prepared Appearance: Prepared medium is light yellow to yellowish-beige, and trace to slightly hazy.

Expected Cultural Response: Cultural response in Nutrient Gelatin incubated at 35 ± 2°C with appropriate atmosphere and examined for growth after 2 - 7 days.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Gelatinase
<i>Bacillus subtilis</i> ATCC® 9372	~10 ⁸	Growth	Positive
<i>Clostridium perfringens</i> ATCC® 13124	~10 ⁸	Growth	Positive
<i>Escherichia coli</i> ATCC® 25922	~10 ⁸	Growth	Negative
<i>Staphylococcus aureus</i> ATCC® 25923	~10 ⁸	Growth	Positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure¹

1. Using a sterile inoculating needle, touch several similar, well-isolated colonies on agar and stab directly down the center of the tube to approximately 10 mm from the bottom.
2. Incubate at 35 ± 2°C for 24 - 48 hours. Incubate uninoculated control tube with the test. Incubation may be extended to 14 days for some organisms.
3. Examine at various intervals. Transfer the tubes to a refrigerator or ice bath. Do not shake the tubes when transferring from incubator to refrigerator. Gently invert the chilled tubes to test for solidity.

Results

Positive: Medium remains liquefied after refrigeration.

Negative: Medium becomes solid after refrigeration.

Uninoculated control tube: Medium becomes solid after refrigeration.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Use this method for detecting gelatinase only if the identification procedure permits incubation beyond 48 hours.
3. Gelatin is liquid at temperatures above 20°C. If tubes are incubated at 35°C, they must be refrigerated in order to read for liquefaction. Include uninoculated tube in the test procedure for comparison.
4. Growth and liquefaction frequently occur only at the tube surface. To prevent a false-negative interpretation, handle tubes carefully when warm so liquefied gelatin remains at the surface of the tube.

Packaging

Nutrient Gelatin	Code No.	7471A	500 g
		7471B	2 kg
		7471C	10 kg

References

1. **Isenberg, H. D. (ed.)**. 1994. Clinical microbiology procedures handbook, Sup. 1. American Society for Microbiology, Washington, D.C.
2. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co. Inc. New York, NY.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.)**. 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.