PRODUCT SPECIFICATION SHEET

Nutrient Gelatin (DM186)

Intended Use
Nutrient Gelatin (DM186) is recommended for detection of gelatin liquefaction by proteolytic microorganisms.

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. Nutrient Gelatin is prepared as per the formulation formerly used in the examination of water, sewage and other materials of sanitary importance.[1] Gelatin liquefaction is one of the characteristics used in the classification of members of the *Enterobacteriaceae* and nonfermenting gram-negative bacteria. The use of Nutrient Gelatin for determining gelatin liquefaction patterns is considered to be the "standard" method for taxonomic studies, since the rate of liquefaction is important in the characterization of groups within the *Enterobacteriaceae* family as well as other groups of microorganisms.[2,3] If the proteolytic enzyme gelatinase is present, gelatin is hydrolyzed and loses its gelling characteristic.[4] Edwards and Ewing consider gelatin liquefaction to be an essential test for differentiation of enteric bacilli.[5] Nutrient Gelatin is used chiefly for identification of pure cultures of bacteria that are not particularly fastidious in regard to nutritional requirements. This medium can also be used for the microbial plate counts of water.

Principles of the Procedure
Nutrient Gelatin contains peptic digest of animal tissue and beef extract which provides carbon, nitrogen and essential nutrients for the growth of non-fastidious organisms. Gelatin is the substrate for the determination of the ability of an organism to produce gelatinase, a proteolytic enzyme active in the liquefaction of gelatin.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	5.00
Beef extract	3.00
Gelatin	120.00
Final pH: 6.8± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 128 grams of the medium in one liter of warm (50°C) distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Mix well and dispense into test tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Allow the tubed medium to cool in an upright position.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing slightly coarse powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in tubes as butts
Reaction of 12.8% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 12.0% Gelatin gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 1 to 7 days, (Incubated anaerobically for *Cl. perfringens*). (For gelatinase test, cool below 20°C)

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Gelatinase
1.	*Clostridium perfringens* ATCC 12924	50 – 100	good-luxuriant	positive reaction
2.	*Bacillus subtilis* ATCC 6633	50 – 100	good-luxuriant	positive reaction
3.	*Escherichia coli* ATCC 25922	50 – 100	good-luxuriant	negative reaction
4.	*Proteus vulgaris* ATCC 13315	50 – 100	good-luxuriant	positive reaction
5.	*Staphylococcus aureus* ATCC 25923	50 – 100	good-luxuriant	positive reaction

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

Test Procedure

1. An 18-24 hours old pure culture from Triple Sugar Iron Agar (DM254) or Kligler Iron Agar (DM127) is stab-inoculated in Nutrient Gelatin with an inoculating needle directly down the centre of the medium to a depth of approximately one half an inches from the bottom of the tube.
2. Incubate the tubes including an un-inoculated control at 35±2°C for 24-48 hours. Many species require prolonged incubation for gelatin liquefaction.
3. Gelatin is solid at 20°C or less temperature and liquid at 35°C or higher temperature. Gelatin liquefies at about 28°C, so incubation is carried out at 35°C but kept in a refrigerator for about 2 hours before interpretation of the results.
4. Liquefaction of gelatin occurs on the surface layer, so care should be taken not to shake the tubes.
5. Control is run along with every testing as gelling ability of gelatin varies and also the gelatin concentration should not exceed 12% as it may inhibit growth.
6. For plate counts of water, the incubation is carried out at 20-22°C for upto 30 days.

Results

1. Examine the tubes for growth (turbidity) and liquefaction at various intervals during the incubation process.
2. Use uninoculated control tubes for comparison.
3. At each interval, tighten caps and transfer the tubes to a refrigerator or ice bath for a sufficient time period to determine whether liquefaction has or has not occurred.
4. It is important that the tubes not be shaken during the transfer from incubator to refrigerator.
5. When reading results, invert the chilled tubes to test for solidification or liquefaction.
6. Positive Gelatinase reaction: Medium remains liquefied after refrigeration.
7. Negative Gelatinase reaction: Medium becomes solid after refrigeration.
8. Uninoculated control tube: Medium becomes solid after refrigeration.

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Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

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

Limitations of the Procedure

1. This medium is not recommended for determination of gelatin liquefaction by fastidious species and obligate anaerobes.
2. 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 an uninoculated tube in the test procedure for comparison.
3. Growth and liquefaction frequently occur only at the surface of the tube. To prevent a false-negative interpretation, handle tubes carefully when warm so that liquified gelatine remains at the surface of the tube.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Nutrient Gelatin
Product Code : DM186
Available Pack sizes : 500gm

References

1. American Public Health Association, 1975, Standard Methods for the Examination of Water and Wastewater, 14th Ed., APHA, Washington, D.C.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
4. Isenberg, H. D. (ed.). 1994. Clinical microbiology procedures handbook, Sup. 1. American Society for Microbiology, Washington, D.C.
5. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.

Further Information

For further information please contact your local MICROMASTER Representative.

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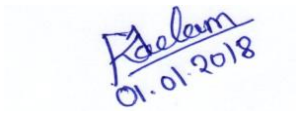	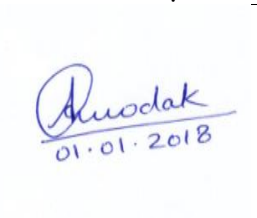	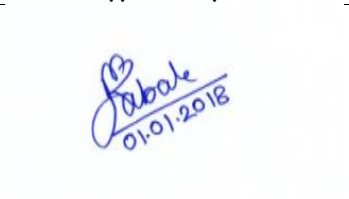
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