

Nutrient Broth

Intended Use

Nutrient Broth is used for the cultivation of many species of nonfastidious microorganisms.

Summary and Explanation

Nutrient Broth has the formula originally designed for use in the *Standard Methods for Examination of Water and Wastewater*. It is not a recommended bacteriological medium in later editions of this publication. It is one of several nonselective media useful in routine cultivation of microorganisms.¹⁻³

Principles of the Procedure

This relatively simple formulation supports the growth of nonfastidious microorganisms due to its content of peptone and beef extract.

User Quality Control

Identity Specifications

Difco™ Nutrient Broth

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 0.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good
<i>Staphylococcus aureus</i>	25923	10 ² -10 ³	Good

Formula

Difco™ Nutrient Broth

Approximate Formula* Per Liter

Beef Extract	3.0	g
Peptone	5.0	g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Dissolve 8 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate tubes of the broth medium with the test samples. Incubate tubes for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

After incubation, growth is evidenced by the appearance of turbidity in the broth. Aliquots of the broth can be used for subculturing to solid media for purification and identification purposes.

References

1. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
3. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Nutrient Broth

	AOAC	BAM	CCAM	COMPF	SMD
Cat. No.	233000	234000	231000	232000	
	Dehydrated – 100 g	Dehydrated – 500 g	Dehydrated – 2 kg	Dehydrated – 10 kg	

BBL™ Nutrient Broth

	BAM	CCAM	COMPF	SMD
Cat. No.	221669			
	Prepared Tubes, 5 mL (K Tubes) – Pkg. of 10			

Nutrient Gelatin

Intended Use

Nutrient Gelatin is used for the detection of gelatin liquefaction by microbial species.

Summary and Explanation

Nutrient Gelatin is made in accordance with the formula 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} Edwards and Ewing consider gelatin liquefaction to be an essential test for differentiation of enteric bacilli.⁴

Nutrient Gelatin is used chiefly for identification of pure cultures of bacteria that are not particularly fastidious in regard to nutritional requirements.

Principles of the Procedure

The peptone and beef extract supply sufficient nutrients for the growth of nonfastidious bacterial species. The gelatin is the substrate for the determination of the ability of an organism to produce gelatinases, which are proteolytic-like enzymes active in the liquefaction of gelatin.

Formula

Difco™ Nutrient Gelatin

Approximate Formula* Per Liter	
Beef Extract	3.0 g
Peptone	5.0 g
Gelatin	120.0 g

*Adjusted and/or supplemented as required to meet performance criteria.

Directions for Preparation from Dehydrated Product

1. Suspend 128 g of the powder in 1 L of purified water.
2. Warm to 50°C to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Using a heavy inoculum (growth from an 18-24 hour pure culture), stab the tubes of Nutrient Gelatin with an inoculat-

ing needle directly down the center of the medium to a depth of approximately one-half an inch from the bottom of the tube. Incubate tubes, including an uninoculated control, at 35 ± 2°C for 24-48 hours and up to 14 days.

Expected Results

At various intervals during the incubation process, examine the tubes for growth (turbidity) and liquefaction. Use uninoculated control tubes for comparison. 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. It is important that the tubes not be shaken during the transfer from incubator to refrigerator. When reading results, invert the chilled tubes to test for solidification or liquefaction.³

Consult appropriate texts for results with specific organisms.³⁻⁶

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 gelatin remains at the surface of the tube.³

References

1. American Public Health Association. 1960. Standard methods for the examination of water and sewage, 9th ed. American Public Health Association, New York, N.Y.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Nutrient Gelatin

USDA

Cat. No. 211100 Dehydrated – 500 g

BBL™ Nutrient Gelatin

USDA

Cat. No. 220974 Prepared Tubes, 8 mL (Deep) – Pkg. of 10

User Quality Control

Identity Specifications

Difco™ Nutrient Gelatin

Dehydrated Appearance:	Tan, fine granular, free-flowing.
Solution:	12.8% solution, soluble in purified water upon warming in a 50-55°C water bath. Solution is light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 12.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Gelatin

Prepare the medium per label directions. Stab inoculate using a heavy inoculum of fresh cultures and incubate at 35 ± 2°C for 1-7 days.

ORGANISM	ATCC™	RECOVERY	GELATINASE
<i>Escherichia coli</i>	25922	Good	–
<i>Staphylococcus aureus</i>	25923	Good	+