

Nutrient Agar

Intended Use

Nutrient Agar is used for the cultivation of bacteria and for the enumeration of organisms in water, sewage, feces and other materials.

Summary and Explanation

Early in the 20th century, the American Public Health Association published the formula for a general purpose medium for the growth of a wide variety of nonfastidious microorganisms.¹ This was in recognition of the need for a standardized medium for the use in the examination of water and wastewater, dairy products and various foods. This relatively simple formulation has stood the test of time, and with the name of Nutrient Agar, is still specified in current compendia of methods for the microbiological examination of a broad spectrum of materials.²⁻⁵ Additionally, it is used in the laboratory for the cultivation and maintenance of nonfastidious species.

User Quality Control

Identity Specifications

Difco™ Nutrient Agar

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in purified water upon boiling. Solution is light to medium amber, clear to slightly opalescent.
Prepared Appearance:	Light amber, very slightly to slightly opalescent.
Reaction of 2.3% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Nutrient Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Enterococcus faecalis</i>	19433	10 ² -10 ³	Good
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good
<i>Pseudomonas aeruginosa</i>	27853	10 ² -10 ³	Good

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Principles of the Procedure

Nutrient Agar consists of peptone, beef extract and agar. This relatively simple formulation provides the nutrients necessary for the replication of a large number of microorganisms that are not excessively fastidious. The beef extract contains water-soluble substances including carbohydrates, vitamins, organic nitrogen compounds and salts. Peptones are the principle sources of organic nitrogen, particularly amino acids and long-chained peptides. Agar is the solidifying agent.

Formula

Difco™ Nutrient Agar

Approximate Formula* Per Liter

Beef Extract	3.0	g
Peptone	5.0	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 23 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute 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

Liquefy the agar if prepared tubes are used, cool to 45-50°C and pour into Petri dishes. Allow to solidify for at least 30 minutes. Use standard procedures to obtain isolated colonies from specimens. Incubate plates at 35 ± 2°C for 18-24 hours and 42-48 hours, if necessary.

Tubed slants are used primarily for the cultivation and maintenance of pure cultures. They should be inoculated with an inoculating loop and incubated under the same conditions as the plated medium.

Expected Results

Examine plates for growth.

Growth from tubes inoculated with pure cultures may be used for biochemical and/or serological testing.

References

1. American Public Health Association. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, New York, N.Y.
2. U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
3. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
4. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. 1. AOAC International, Gaithersburg, Md.
5. 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 Agar

	AOAC	BAM	CCAM	COMPF	ISO	SMWW	USDA
Cat. No.	212000						
	213000						
	211665						

BBL™ Nutrient Agar

	AOAC	BAM	CCAM	COMPF	ISO	SMWW	USDA
<i>United States and Canada</i>							
Cat. No.	297801						
	220968						
	220971						

Europe

Cat. No. 256035 Prepared Plates – Pkg. of 20*

Mexico

Cat. No. 257500 Prepared Plates – Pkg. of 10*

*Store at 2-8°C.



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

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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					Dehydrated – 100 g
234000					Dehydrated – 500 g
231000					Dehydrated – 2 kg
232000					Dehydrated – 10 kg

BBL™ Nutrient Broth

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