

References

1. Koser. 1923. J. Bacteriol. 8:493.
2. Simmons. 1926. J. Infect. Dis. 39:209.
3. Trabulsi and Ewing. 1962. Public Health Lab. 20:137.
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. Farmer. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ Acetate Differential Agar

BAM **COMPF** **SMD**

Cat. No. 274210 Dehydrated – 500 g

BBL™ Acetate Differential Agar

BAM **COMPF** **SMD**

Cat. No. 221375 Prepared Slants – Pkg. of 10

Acidicase™ Peptone

(See *Casamino Acids*)

Actinomyces Broth

Intended Use

Actinomyces Broth is used as a liquid medium or, with the addition of 7 or 20 g/L of agar, as a semisolid or solid medium, respectively, for the maintenance or cultivation of *Actinomyces* species.

Summary and Explanation

Actinomyces Broth is a basic medium modified from the Actinomyces Maintenance Medium of Pine and Watson.¹ It is recommended for use in the growth and maintenance of members of the genus *Actinomyces*.²

Principles of the Procedure

Actinomyces Broth contains meat infusion, peptone, yeast extract, soluble starch, L-cysteine and dextrose, which provide carbon, nitrogen, sulfur, vitamins and other growth factors required for the metabolism of *Actinomyces* spp. The salts provide essential minerals and electrolytes.

User Quality Control

Identity Specifications

BBL™ Actinomyces Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	5.7% solution, soluble in purified water. Solution is light to medium, yellow to tan, trace hazy to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, trace hazy to moderately hazy.
Reaction of 5.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

BBL™ Actinomyces Broth

Prepare the medium per label directions. Inoculate and incubate anaerobically at 35 ± 2°C for 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RESULT
<i>Actinomyces bovis</i>	13683	<10 ³	Growth
<i>Actinomyces israelii</i>	10049	<10 ³	Growth

Formula

BBL™ Actinomyces Broth

Approximate Formula* Per Liter		
Heart Muscle, Infusion from (solids)	2.0	g
Pancreatic Digest of Casein	17.0	g
Yeast Extract	10.0	g
Sodium Chloride	5.0	g
Dipotassium Phosphate	13.0	g
Monopotassium Phosphate	2.0	g
Dextrose	5.0	g
Ammonium Sulfate	1.0	g
L-Cysteine HCl	1.0	g
Soluble Starch	1.0	g
Magnesium Sulfate	0.2	g
Calcium Chloride	0.01	g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 57 g of the powder in 1 L of purified water. Add agar, 7 or 20 g/L, if a semisolid or solid medium is desired.
2. If agar is added, heat with frequent agitation just until solution occurs.
3. Dispense and autoclave at 121°C for 10 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate *Actinomyces* cultures into tubes containing broth, semisolid or solid media. The semisolid medium should be stab-inoculated and the slanted medium should be inoculated over its entire surface.

Incubate cultures at 35 ± 2°C in an anaerobic atmosphere (BBL™ GasPak™ EZ anaerobic system, or alternative system for the cultivation of anaerobic microorganisms).

Expected Results

After growth is obtained, tubes containing broth may be frozen for long-term storage. Cultures grown in the semisolid medium can be refrigerated after growth has been obtained. Agar slant cultures are for use in a relatively short period of time.

References

1. Pine and Watson. 1959. J. Lab. Clin. Med. 54:107.
2. Ajello, Georg, Kaplan and Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994. U.S. Government Printing Office, Washington, D.C.

Availability

BBL™ Actinomycetes Broth

Cat. No. 210920 Dehydrated – 500 g

Actinomycete Isolation Agar Glycerol

Intended Use

Actinomycete Isolation Agar is used with added glycerol for isolating and cultivating actinomycetes from soil and water.

Glycerol is used in preparing microbiological culture media.

Summary and Explanation

Although some genera are important to human medicine, most of the actinomycetes are part of the indigenous flora of soil, water and vegetation. Actinomycetes may impart a musty odor to water or a muddy flavor to fish.¹ Actinomycetes can cause massive growths which will form a thick foam in the activated sludge process, causing a disruption in wastewater treatment.^{2,3} Actinomycetes are gram-positive, acid-fast cells, growing as filaments that may branch and may form irregularly shaped rods and cocci.

Olsen⁴ formulated Actinomycete Isolation Agar for isolating and cultivating actinomycetes from soil and water. The formula is supplemented with glycerol, a highly purified fermentable alcohol used occasionally for differentiating certain bacteria and in media for isolating and culturing fastidious bacteria.

Principles of the Procedure

Actinomycete Isolation Agar contains sodium caseinate which is a source of nitrogen. Asparagine is an amino acid and a

source of organic nitrogen. Sodium propionate is a substrate used in anaerobic fermentation. Dipotassium phosphate provides buffering capability to maintain pH balance. Magnesium sulfate and ferrous sulfate provide sources of sulfates and metallic ions. Agar is the solidifying agent. The added glycerol is a source of carbon.

Formulae

Difco™ Actinomycete Isolation Agar

Approximate Formula* Per Liter

Sodium Caseinate	2.0	g
Asparagine	0.1	g
Sodium Propionate	4.0	g
Dipotassium Phosphate	0.5	g
Magnesium Sulfate	0.1	g
Ferrous Sulfate	1.0	mg
Agar	15.0	g

Difco™ Glycerol

Glycerin

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

Directions for Preparation from Dehydrated Product

1. Suspend 22 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. Add 5 g of Glycerol.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate medium and incubate at 30°C for up to 72 hours.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
2. Lechevalier. 1975. Actinomycetes of sewage-treatment plants. Environ. Protection Technol. Ser., EPA-600/2-75-031, U. S. Environmental Protection Agency, Cincinnati, Ohio.
3. Lechevalier and Lechevalier. 1974. Int. J. Syst. Bacteriol. 24:278.
4. Olsen. 1960. Personal communication.

Availability

Difco™ Actinomycete Isolation Agar

Cat. No. 212168 Dehydrated – 500 g

Difco™ Glycerol

Cat. No. 228210 Bottle – 100 g
228220 Bottle – 500 g

User Quality Control

Identity Specifications

Difco™ Actinomycete Isolation Agar

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 2.2% solution, soluble in purified water upon boiling with 0.5% Glycerol. Solution is light to medium amber, opalescent to opaque with precipitation.

Prepared Appearance: Medium amber, opalescent.

Reaction of 2.2% Solution with 0.5% Glycerol at 25°C: pH 8.1 ± 0.2

Cultural Response

Difco™ Actinomycete Isolation Agar

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Streptomyces achromogenes</i>	12767	10 ² -10 ³	Good
<i>Streptomyces albus</i>	3004	10 ² -10 ³	Good
<i>Streptomyces lavendulae</i>	8664	10 ² -10 ³	Good