

Acetate Differential Agar

Intended Use

Acetate Differential Agar is used for the differentiation of *Shigella* species from *Escherichia coli*.

Summary and Explanation

Organic acids have been used widely as an aid to the differentiation of *Enterobacteriaceae*, usually in formulae that contained organic nitrogen sources. Most bacteria, however, can use citrate and acetate in the presence of organic nitrogen.

The citrate media of Koser¹ and Simmons² were free of organic nitrogen and, therefore, were a true measure of citrate utilization. In a further extension of this approach, Trabulsi and Ewing developed Acetate Differential Agar, a chemically defined medium utilizing sodium acetate that enables the differentiation of *Shigella* spp. from *E. coli*, particularly anaerogenic, nonmotile biotypes.^{3,4} Their basal medium was Simmons Citrate Agar in which sodium acetate was substituted for sodium citrate.

Principles of the Procedure

Acetate Differential Agar consists of a mixture of salts and sodium acetate, as a sole source of carbon, in a chemically defined medium devoid of organic nitrogen.

Typical cultures of *Shigella* are unable to utilize acetate and fail to grow; therefore, the medium remains unchanged. Most cultures of *E. coli* and closely related organisms grow well within 24-48 hours, but some strains grow more slowly and a few cannot use the acetate as a source of carbon. The blue color of the bromthymol blue is due to the production of alkaline products from the utilization of the sodium acetate.

Formula

Difco™ Acetate Differential Agar

Approximate Formula* Per Liter		
Sodium Acetate	2.0	g
Magnesium Sulfate	0.1	g
Sodium Chloride	5.0	g
Monoammonium Phosphate	1.0	g
Dipotassium Phosphate	1.0	g
Bromthymol Blue	0.08	g
Agar	20.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 29.2 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. Dispense into tubes to allow a 10 mm butt and a 30 mm slant.
4. Autoclave at 121°C for 15 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the agar slant surfaces with pure cultures of unknown organisms. Incubate all tubes for up to 7 days at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Bacteria capable of utilizing acetate as the sole carbon source will grow on the medium and produce an alkaline reaction (blue color). For a listing of organisms capable of utilizing acetate, consult appropriate texts.⁴⁻⁶

Limitations of the Procedure

Some strains of *E. coli* utilize acetate slowly or not at all and may give a false-negative reaction. Sodium acetate is utilized as a sole source of carbon by some biotypes of *S. flexneri* 4a.⁴

User Quality Control

Identity Specifications

Difco™ Acetate Differential Agar

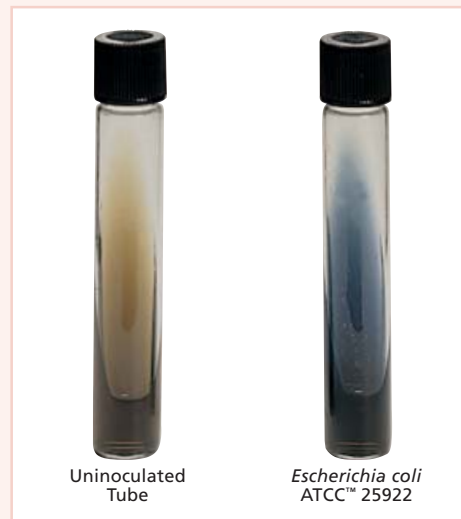
Dehydrated Appearance:	Medium yellowish-tan to light green, free-flowing, homogeneous.
Solution:	2.92% solution, soluble in purified water upon boiling. Solution is emerald green, slightly opalescent.
Prepared Appearance:	Emerald green to green, slightly opalescent.
Reaction of 2.92% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Difco™ Acetate Differential Agar

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 2-7 days. Acetate utilization is indicated by a color change of the slant from green to blue.

ORGANISM	ATCC™	RECOVERY	ACETATE UTILIZATION
<i>Escherichia coli</i>	25922	Good	Positive (blue)
<i>Shigella sonnei</i>	25931	Poor to good	Negative (green)



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 **COMP** **F** **SMD**

Cat. No. 274210 Dehydrated – 500 g

BBL™ Acetate Differential Agar

BAM **COMP** **F** **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.

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.

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