User Quality Control

Identity Specifications

Difco™ Malonate Broth

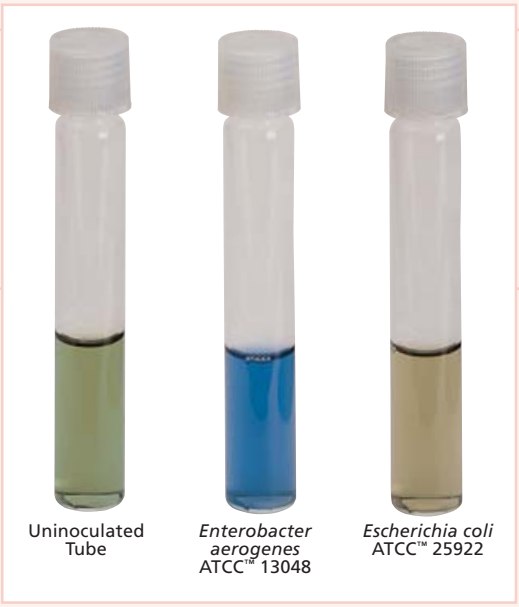
Dehydrated Appearance:	Light green, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water. Solution is green, clear.
Prepared Appearance:	Green, clear.
Reaction of 0.8% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Malonate Broth

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

ORGANISM	ATCC™	MEDIUM COLOR
Enterobacter aerogenes	13048	Blue
Enterobacter cloacae	13047	Blue
Escherichia coli	25922	Green
Klebsiella pneumoniae	13883	Blue
Salmonella choleraesuis subsp. *arizonae*	13314	Blue
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	Green



Expected Results

Malonate utilization is indicated by a change in the color of the medium from green to blue:

Positive: Blue
Negative: Green

Limitation of the Procedure

A slight bluing (blue-green) of the medium may occur after prolonged incubation.[5] In such cases, care should be taken in interpreting results.

References

1. Leifson. 1933. J. Bacteriol. 26:329.
2. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.
3. Marshall (ed.). 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. Edwards and Ewing. 1962. *Enterobacteriaceae*. U.S. Public Health Service Bulletin No. 734:19.
5. Oberhofer. 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, N.Y.

Availability

Difco™ Malonate Broth

COMPF SMD

Cat. No. 239520 Dehydrated – 500 g

Malonate Broth, (Ewing) Modified

Intended Use

Malonate Broth, as modified by Ewing, is used for the differentiation of coliforms and other enteric organisms.

Summary and Explanation

Leifson, in 1933, developed a synthetic liquid medium which differentiated *Aerobacter* (now *Enterobacter*) from *Escherichia* species based on their ability to utilize malonate.[1] The modification, in which dextrose and yeast extract are incorporated, was devised by Ewing et al.[2]

The addition of yeast extract, a source of vitamins, and a relatively small amount of dextrose, a minimal carbon source, is included in Ewing's modification to stimulate the growth of some organisms. The medium, therefore, will support the growth of organisms that cannot utilize malonate or ammonium salt, but any spontaneous alkalinization produced by such organisms is buffered by the phosphate system and counteracted by the acid produced in the fermentation of the small amount of dextrose.[3] An alkaline result (blue color) is only produced in this medium by organisms capable of utilizing malonate and ammonium sulfate.

Principles of the Procedure

An organism that simultaneously can utilize sodium malonate as its carbon source and ammonium sulfate as its nitrogen source produces an alkalinity due to the formation of sodium hydroxide.[3] The alkali changes the color of the bromthymol blue indicator in the medium to light blue to Prussian blue. The color of the medium remains unchanged in the presence of an organism that cannot utilize these substances. Some malonate-negative strains produce a yellow color due to the fermentation of dextrose only, which results in increased acidity causing the pH indicator to change to yellow at a pH of 6.0.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Malonate Broth, Modified

Dehydrated Appearance:	Beige to light green, free-flowing, homogeneous.
Solution:	0.93% solution, soluble in purified water with agitation. Solution is green, clear.
Prepared Appearance:	Green, clear.
Reaction of 0.93% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Malonate Broth, Modified

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

ORGANISM	ATCC™	RECOVERY	MEDIUM COLOR
Enterobacter aerogenes	13048	Good	Blue
Enterobacter cloacae	13047	Good	Blue
Escherichia coli	25922	Good	Green
Klebsiella pneumoniae	13883	Good	Blue
Salmonella choleraesuis subsp. *arizonae*	13314	Good	Blue
Salmonella choleraesuis subsp. *choleraesuis* serotype Typhimurium	14028	Good	Green

Identity Specifications

BBL™ Malonate Broth, Ewing Modified

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	0.93% solution, soluble in purified water. Solution is light to medium, green, with or without a tint of yellow or blue, clear to slightly hazy.
Prepared Appearance:	Light to medium, green, with or without a tint of yellow or blue, clear to slightly hazy.
Reaction of 0.93% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

BBL™ Malonate Broth, Ewing Modified

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	RECOVERY	MEDIUM COLOR
Enterobacter aerogenes	13048	Good	Blue
Escherichia coli	25922	Good	Yellow-green to gray-green

Formulae

Difco™ Malonate Broth, Modified

Approximate Formula* Per Liter

Yeast Extract	1.0	g
Ammonium Sulfate	2.0	g
Dipotassium Phosphate	0.6	g
Monopotassium Phosphate	0.4	g
Sodium Chloride	2.0	g
Sodium Malonate	3.0	g
Dextrose	0.25	g
Bromthymol Blue	25.0	mg

BBL™ Malonate Broth, Ewing Modified

Approximate Formula* Per Liter

Yeast Extract	1.0	g
Ammonium Sulfate	2.0	g
Dipotassium Phosphate	0.6	g
Monopotassium Phosphate	0.4	g
Sodium Chloride	2.0	g
Sodium Malonate	3.0	g
Dextrose	0.25	g
Bromthymol Blue	25.0	mg

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

Directions for Preparation from Dehydrated Product

1. Dissolve 9.3 g of the powder in 1 L of purified water.
2. Dispense and autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate tubes, using a light inoculum, with growth from an 18- to 24-hour pure culture. Incubate tubes with loosened caps for 18-48 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Bacterial genera in which the majority of species yield a positive alkaline reaction (light blue to Prussian blue color throughout the medium) include:

Enterobacter
Klebsiella
Citrobacter

Genera in which the majority of species yield a negative reaction (color of medium is unchanged or yellow) include:

Escherichia
Salmonella
Shigella
Edwardsiella
Yersinia
Serratia
Morganella
Proteus
Providencia

Limitation of the Procedure

Some malonate-positive organisms produce only slight alkalinity. Compare any tube in question with an uninoculated malonate tube. Any trace of blue color after a 48-hour incubation period denotes a positive test. Before making a final negative interpretation, be sure that test tubes have been incubated for 48 hours.[3]

References

1. Leifson. 1933. J. Bacteriol. *26*:329.
2. Ewing, Davis and Reavis. 1957. Public Health Lab. *15*:153.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ Malonate Broth, Modified

AOAC BAM COMPF SMD USDA

Cat. No. 256910 Dehydrated – 500 g

BBL™ Malonate Broth, Ewing Modified

AOAC BAM COMPF SMD USDA

Cat. No. 211399 Dehydrated – 500 g
221322 Prepared Tubes – Pkg. of 10

Malt Agar

Intended Use

Malt Agar is used for isolating and cultivating yeasts and molds from food and for cultivating yeast and mold stock cultures.

Summary and Explanation

Malt media for yeasts and molds have been widely used for many years. In 1919, Reddish[1] prepared a satisfactory substitute for beer wort from malt extract. Thom and Church[2] used Reddish's medium for their studies of the aspergilli. Malt Agar was also employed by Fullmer and Grimes[3] for their studies of the growth of yeasts on synthetic media. Malt Agar is included in *Official Methods of Analysis of AOAC International*.[4]

Principles of the Procedure

Malt Agar contains malt extract which provides the carbon, protein and nutrient sources required for the growth of microorganisms. Agar is the solidifying agent. The acidic pH of Malt Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

Formula

Difco™ and BBL™ Malt Agar

Approximate Formula* Per Liter

Malt Extract	30.0	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 45 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.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Malt Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, very slightly to slightly opalescent.
Reaction of a 4.5% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

Difco™ Malt Agar

Prepare the medium per label directions. For specific quantities of sterile 1:10 dilution of lactic acid, USP (85%) to add to 100 mL of medium to obtain a pH of 3.5 or 4.5, see the Certificate of Analysis for each lot.* Inoculate and incubate at 30 ± 2°C for 42-48 hours (up to 72 hours if necessary).

**For Certificates of Analysis from Technical Services, phone 800-638-8663, fax 410-527-0244 or via the internet at www.bd.com/regdocs.*

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	10^2-10^3	Good
Candida albicans	10231	10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^2-10^3	Good

Identity Specifications

BBL™ Malt Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	4.5% solution, soluble in purified water upon boiling. Solution is medium to dark, yellow to tan, trace hazy to hazy.
Prepared Appearance:	Medium to dark, yellow to tan, trace hazy to hazy.
Reaction of 4.5% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

BBL™ Malt Agar

Prepare the medium per label directions. Inoculate pour plates with *Saccharomyces cerevisiae* and incubate at 25 ± 2°C for 42-48 hours. Inoculate tubes with other test organisms and incubate at 25 ± 2°C for 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	30-300	Good
Candida albicans	60193	30-300	Good
Saccharomyces cerevisiae	9763	30-300	Good