Malonate Broth

Intended Use

Malonate Broth is used for differentiating *Enterobacter* from *Escherichia* based on malonate utilization.

Summary and Explanation

Malonate Broth, prepared according to the formula described by Leifson,[1] is a liquid medium containing ammonium sulfate as the only source of nitrogen and malonate as the only source of carbon. Leifson was able to demonstrate that the *Enterobacter* group utilizes malonate whereas the *Escherichia* group is unable to grow on the medium.

Malonate Broth is further described for differentiating *Enterobacteriaceae* in food and dairy products.[2,3] More often, the medium referenced is the modified Edwards and Ewing[4] formulation that contains yeast extract and dextrose. The modification permits growth of organisms that would otherwise fail on the unsupplemented Leifson medium.

User Quality Control

Identity Specifications

Difco™ Malonate Broth

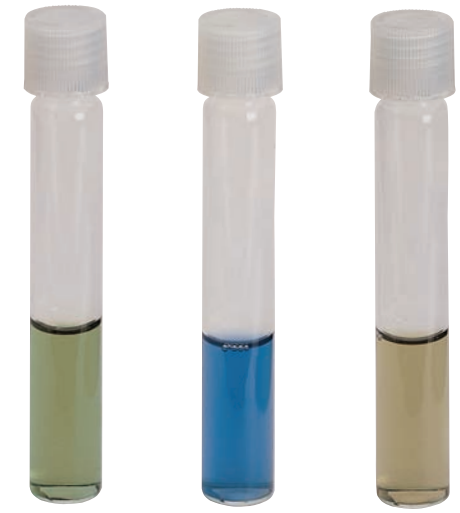
Dehydrated Appearance:	Tan to green, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water. Solution is green, clear.
Prepared Appearance:	Green, clear.
Reaction of 0.8% Solution at 25°C:	pH 6.7 ± 0.2

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 enterica subsp. *arizonae*	13314	Blue
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	Green



Uninoculated Tube

Enterobacter aerogenes ATCC™ 13048

Escherichia coli ATCC™ 25922

Principles of the Procedure

Malonate Broth contains ammonium sulfate, which is the sole source of nitrogen in the medium; sodium malonate is the sole source of carbon. Dipotassium phosphate and monopotassium phosphate provide buffering capability. Sodium chloride maintains the osmotic balance of the medium. Increased alkalinity resulting from malonate utilization causes the indicator, bromthymol blue, to change color from green to blue.

Formula

Difco™ Malonate Broth

Approximate Formula* Per Liter		
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
Bromthymol Blue	25.0	mg

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

Directions for Preparation from Dehydrated Product

1. Suspend 8 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. Avoid introducing extraneous carbon and nitrogen.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

1. Inoculate tubes with a loopful of test organism.
2. Incubate at 35 ± 2°C for 18-48 hours.
3. Examine tubes for a change in the color of the medium from green to blue.

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. Wehr and Frank (ed.). 2004. Standard methods for the examination of dairy products, 17th 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