

# 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.<sup>1</sup> The modification, in which dextrose and yeast extract are incorporated, was devised by Ewing et al.<sup>2</sup>

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 alkalization 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.<sup>3</sup> 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.<sup>3</sup> 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.

## Formula

### 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.

## User Quality Control

### 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
<i>Enterobacter aerogenes</i>	13048	Good	Blue
<i>Escherichia coli</i>	25922	Good	Yellow-green to gray-green

## 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:

<i>Escherichia</i>	<i>Serratia</i>
<i>Salmonella</i>	<i>Morganella</i>
<i>Shigella</i>	<i>Proteus</i>
<i>Edwardsiella</i>	<i>Providencia</i>
<i>Yersinia</i>	

## 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.<sup>3</sup>

## 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

### BBL™ Malonate Broth, Ewing Modified

AOAC BAM COMPF SMD

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