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. In such cases, care should be taken in interpreting results.

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. The modification, in which dextrose and yeast extract are incorporated, was devised by Ewing et al.

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. 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. 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.

References

Availability
Difco™ Malonate Broth
Cat. No. 239520 Dehydrated – 500 g
**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, homogenous.

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.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>RECOVERY</th>
<th>MEDIUM COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter aerogenes</td>
<td>13048</td>
<td>Good Blue</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>13047</td>
<td>Good Blue</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>Good Green</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>13883</td>
<td>Good Blue</td>
<td></td>
</tr>
<tr>
<td>Salmonella choleraesuis subsp. arizonae</td>
<td>13314</td>
<td>Good Blue</td>
<td></td>
</tr>
<tr>
<td>Salmonella choleraesuis serotype Typhimurium</td>
<td>14028</td>
<td>Good Green</td>
<td></td>
</tr>
</tbody>
</table>

### Formulae

**Difco™ Malonate Broth, Modified**

Approximate Formula* Per Liter

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast Extract</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Ammonium Sulfate</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
<td>0.6 g</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
<td>0.4 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium Malonate</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Bromthymol Blue</td>
<td>25.0 mg</td>
</tr>
</tbody>
</table>

**BBL™ Malonate Broth, Ewing Modified**

Approximate Formula* Per Liter

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Yeast Extract</td>
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</tr>
<tr>
<td>Sodium Chloride</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Sodium Malonate</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Dextrose</td>
<td>0.25 g</td>
</tr>
<tr>
<td>Bromthymol Blue</td>
<td>25.0 mg</td>
</tr>
</tbody>
</table>

*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**
- **Serratia**
- **Salmonella**
- **Morganella**
- **Shigella**
- **Proteus**
- **Edwardsiella**
- **Providencia**
- **Yersinia**

### 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.
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 \textit{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
\textbf{Difco™ and BBL™ Malt Agar}

\begin{align*}
\text{Approximate Formula* Per Liter} & \\
\text{Malt Extract} & \text{30.0 g} \\
\text{Agar} & \text{15.0 g}
\end{align*}

*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 \textit{Difco}™ and \textit{BBL}™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications
\textbf{Difco™ Malt Agar}

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

\textbf{Cultural Response}

\begin{align*}
\text{Difco™ Malt Agar} & \\
\text{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).}
\end{align*}

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

\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{ORGANISM} & \textbf{ATCC} & \textbf{INOCULUM CFU} & \textbf{RECOVERY} \\
\hline
Aspergillus niger & 16404 & 10^{-10} & Good \\
Candida albicans & 10231 & 10^{-10} & Good \\
Saccharomyces cerevisiae & 9763 & 10^{-10} & Good \\
\hline
\end{tabular}

Identity Specifications
\textbf{BBL™ Malt Agar}

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

\textbf{Cultural Response}

\begin{align*}
\text{BBL™ Malt Agar} & \\
\text{Prepare the medium per label directions. Inoculate pour plates with \textit{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.}
\end{align*}

\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{ORGANISM} & \textbf{ATCC} & \textbf{INOCULUM CFU} & \textbf{RECOVERY} \\
\hline
Aspergillus niger & 16404 & 30-300 & Good \\
Candida albicans & 60193 & 30-300 & Good \\
Saccharomyces cerevisiae & 9763 & 30-300 & Good \\
\hline
\end{tabular}