**MacConkey Agars with Sorbitol**

**MacConkey Sorbitol Agar • MacConkey II Agar with Sorbitol • Sorbitol MacConkey II Agar with Cefixime and Tellurite**

### Intended Use

MacConkey Sorbitol Agar and MacConkey II Agar with Sorbitol are selective and differential media for the detection of sorbitol-nonfermenting *Escherichia coli* serotype O157:H7 associated with hemorrhagic colitis. These media are also referred to as “Sorbitol MacConkey Agar.”

Sorbitol MacConkey II Agar with Cefixime and Tellurite (SMAC-CT) is a more selective and differential medium designed to inhibit *Proteus mirabilis*, non-O157 *E. coli* strains and other sorbitol-nonfermenting strains.

### Summary and Explanation

*Escherichia coli* serotype O157:H7 is a human pathogen associated with hemorrhagic colitis that results from the action of a shiga-like toxin (SLT). On standard MacConkey Agar containing lactose, this strain is indistinguishable from other lactose-fermenting *E. coli*. Unlike most *E. coli* strains, *E. coli* O157:H7 ferments sorbitol slowly or not at all. Therefore, the efficacy of MacConkey Agar containing sorbitol instead of lactose as a differential medium for the detection of *E. coli* O157:H7 in stool cultures was determined. Field trial results showed that the growth of *E. coli* O157:H7 on MacConkey Agar with Sorbitol was heavy and occurred in almost pure culture as colorless sorbitol-nonfermenting colonies. Most organisms

#### 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™ MacConkey Sorbitol Agar**

| Dehydrated Appearance: | Pinkish beige, free-flowing, homogeneous (may contain dark particles). |
| Solution: | 5.0% solution, soluble in purified water upon boiling. Solution is reddish-purple, very slightly to slightly opalescent. |
| Prepared Appearance: | Reddish-purple, slightly opalescent. |
| Reaction of 5.0% Solution at 25°C: | pH 7.1 ± 0.2 |

**Cultural Response**

**Difco™ MacConkey Sorbitol Agar**

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>COLONY COLOR</th>
<th>BILE PPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>29212</td>
<td>10^2-2 x 10^3</td>
<td>Marked to complete inhibition</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>10^2-10^3</td>
<td>Good</td>
<td>Pink-red</td>
<td>+</td>
</tr>
<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>35150</td>
<td>10^2-10^3</td>
<td>Good</td>
<td>Colorless</td>
<td>–</td>
</tr>
</tbody>
</table>

**Identity Specifications**

**BBL™ MacConkey II Agar with Sorbitol**

| Dehydrated Appearance: | Fine, homogeneous, may contain dark particles. |
| Solution: | 5.0% solution, soluble in purified water upon boiling. Solution is medium to dark, rose to brown rose, with or without a trace orange tint, clear to slightly hazy. |
| Prepared Appearance: | Medium to dark, rose to brown-rose, with or without a trace orange tint, clear to slightly hazy. |
| Reaction of 5.0% Solution at 25°C: | pH 7.1 ± 0.2 |

**Cultural Response**

**BBL™ MacConkey II Agar with Sorbitol**

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (42-48 hours for *E. coli* ATCC™ 25922).

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>COLONY COLOR</th>
<th>BILE PPT</th>
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</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>10^2-10^4</td>
<td>Good</td>
<td>Pink to red to rose-red</td>
<td>+</td>
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<tr>
<td><em>Escherichia coli</em> O157:H7</td>
<td>35150</td>
<td>10^2-10^4</td>
<td>Good</td>
<td>Colorless</td>
<td>–</td>
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<tr>
<td><em>Proteus mirabilis</em></td>
<td>12453</td>
<td>10^2-10^4</td>
<td>Good</td>
<td>Colorless</td>
<td>–</td>
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</table>
of the fecal flora ferment sorbitol and appear pink on this medium. MacConkey Agar with Sorbitol, therefore, permits ready recognition of E. coli O157:H7 in stool cultures.1,3

The addition of cefxime and tellurite significantly reduces the number of sorbitol nonfermenters that need to be screened during the attempted isolation of E. coli O157:H7.4,5

**Principles of the Procedure**

MacConkey Sorbitol Agar and MacConkey II Agar with Sorbitol, modified MacConkey agars using sorbitol instead of lactose, are only slightly selective, since the concentration of bile salts, which inhibits gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci. MacConkey II Agar with Sorbitol is also formulated to reduce swarming of Proteus species.

Differentiation of enteric microorganisms is achieved by the combination of sorbitol and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate sorbitol.

SMAC-CT is modified MacConkey II Agar using sorbitol instead of lactose and with cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) added. Cefxime inhibits Proteus spp. and tellurite inhibits non-O157 E. coli and other organisms, thus improving the selectivity of SMAC-CT for E. coli O157:H7.

**Formulae**

**Difco™ MacConkey Sorbitol Agar**

Approximate Formula* Per Liter  
Peptone ................................................................. 15.5 g  
Proteose Peptone ................................................... 3.0 g  
D-Sorbitol ............................................................ 10.0 g  
Bile Salts .............................................................. 1.5 g  
Sodium Chloride .................................................. 5.0 g  
Agar ................................................................. 15.0 g  
Neutral Red ......................................................... 0.03 g  
Crystal Violet ....................................................... 1.0 mg

**BBL™ MacConkey II Agar with Sorbitol**

Approximate Formula* Per Liter  
Pancreatic Digest of Gelatin .................................... 17.0 g  
Pancreatic Digest of Casein .................................... 1.5 g  
Peptic Digest of Animal Tissue ................................. 1.5 g  
D-Sorbitol ............................................................ 10.0 g  
Bile Salts .............................................................. 1.5 g  
Sodium Chloride .................................................. 5.0 g  
Agar ................................................................. 13.5 g  
Neutral Red ......................................................... 0.03 g  
Crystal Violet ....................................................... 1.0 mg

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

**Directions for Preparation from Dehydrated Product**

1. Suspend 50 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. Test samples of the finished product for performance using stable, typical control cultures.

**Procedure**

Prepare plated medium from tubed agar deeps by liquefying the medium in boiling water, cooling to 45-50°C and pouring into sterile Petri dishes.

Use standard procedures to obtain isolated colonies from specimens. Incubate plates, protected from light, in an inverted position (agar side up) at 35 ± 2°C for 18-24 hours in an aerobic atmosphere without additional CO₂.

**Expected Results**

After 18-24 hours of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Sorbitol fermenters produce pink to red colonies, some surrounded by zones of precipitated bile, while sorbitol nonfermenters produce colorless colonies.

Most fecal flora will be partially to completely inhibited on SMAC-CT.

Gram staining, biochemical tests and serological procedures should be performed to confirm findings.

**Limitations of the Procedure**

1. It has been reported that some *Enterobacteriaceae* and *Pseudomonas aeruginosa* are inhibited on MacConkey Agar when incubated in a CO₂-enriched atmosphere.4
2. Prolonged incubation of the culture may result in colonies of *E. coli* serotype O157:H7 losing their characteristic colorless appearance. There are additional species of facultatively anaerobic gram-negative rods that do not ferment sorbitol.
3. The color of sorbitol-positive colonies can fade, making them hard to distinguish from sorbitol-negative colonies.7

**References**

Availability

**Difco™ MacConkey Sorbitol Agar**

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<tr>
<th>Code</th>
<th>Description</th>
<th>Cat. No.</th>
<th>Measurement</th>
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<td>279100</td>
<td>Dehydrated – 500 g</td>
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**BBL™ MacConkey II Agar with Sorbitol**

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**United States and Canada**

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**Japan**

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**BBL™ MacConkey II Agar with Sorbitol/SS Agar**

**Japan**

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**BBL™ Sorbitol MacConkey II Agar with Cefixime and Tellurite**

**United States and Canada**

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*Store at 2-8°C.*