**m FC Agar • m FC Broth Base**

**Rosolic Acid**

**Intended Use**

m FC Agar and m FC Broth Base are used with Rosolic Acid in cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

**Summary and Explanation**

Geldreich et al. formulated a medium to enumerate fecal coliforms (MFC) using the membrane filter (MF) technique without prior enrichment. Fecal coliforms (i.e., those found in the feces of warm-blooded animals) are differentiated from coliforms from environmental sources by their ability to grow at 44.5 ± 0.5°C.

Many “standard methods” membrane filtration procedures specify m FC medium for testing water. The American Public Health Association (APHA) specifies m FC medium and incubation at 44.5 ± 0.5°C in the fecal coliform membrane filter procedure, the delayed-incubation fecal coliform procedure and the two-layer agar method for recovering injured fecal coliforms. AOAC International specifies m FC Agar for detecting total coliforms and fecal coliforms in foods.

The U. S. Environmental Protection Agency specifies using m FC medium in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF method.

**Principles of the Procedure**

m FC Agar and m FC Broth Base contain peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins that stimulate bacterial growth. Lactose is a carbohydrate. Bile Salts No. 3 inhibits growth of gram-positive bacteria. m FC Agar contains agar as the solidifying agent. The differential indicator system combines aniline blue and rosolic acid.

### User Quality Control

#### Identity Specifications

**Difco™ m FC Agar**

| Dehydrated Appearance: | Beige with slight blue tint, free-flowing, homogeneous. |
| Solution: | 5.2% solution, soluble in purified water upon boiling. Without 1% Rosolic Acid: blue, very slightly to slightly opalescent, may have a slight precipitate. With 1% Rosolic Acid: cranberry red, slightly opalescent, may have a slight precipitate. |
| Prepared Appearance: | Without 1% Rosolic Acid—Blue, slightly opalescent. With 1% Rosolic Acid—Cranberry red, slightly opalescent. |
| Reaction of 5.2% Solution at 25°C: | pH 7.4 ± 0.2 (without 1% Rosolic Acid) |

**Difco™ m FC Broth Base**

| Dehydrated Appearance: | Beige with slight blue tint, free-flowing, homogeneous. |
| Solution: | 3.7% solution, soluble in purified water upon boiling. Solution is blue, slightly opalescent, may have a precipitate. |
| Prepared Appearance: | Without 1% Rosolic Acid—Blue, slightly opalescent. With 1% Rosolic Acid—Cranberry red, slightly opalescent, may have a very fine precipitate. |
| Reaction of 3.7% Solution at 25°C: | pH 7.4 ± 0.2 (without 1% Rosolic Acid) |

**Difco™ Rosolic Acid**

| Dehydrated Appearance: | Dark reddish-brown with metallic green particles, free-flowing, fine crystalline powder. |
| Solution: | 1.0% solution, soluble in 0.2N NaOH. Solution is deep red, clear to very slightly opalescent. |

#### Cultural Response

**Difco™ m FC Agar or m FC Broth Base**

Prepare the medium per label directions with 1% Rosolic Acid. Using the membrane filter technique, inoculate and incubate plates at 44.5 ± 0.5°C for 24 ± 2 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC®</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>COLONY COLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>19433</td>
<td>10^3–2×10^4</td>
<td>Marked to complete inhibition</td>
<td>–</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>20–80</td>
<td>Good</td>
<td>Blue</td>
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</tbody>
</table>

**Escherichia coli ATCC® 25922**
Colonies of fecal coliforms are blue; non-fecal coliforms and other organisms are gray to cream-colored.

**Formulae**

**Difco™ m FC Agar**

Approximate Formula* Per Liter

- Tryptose ........................................... 10.0 g
- Proteose Peptone No. 3 .......................... 5.0 g
- Yeast Extract ..................................... 3.0 g
- Lactose ........................................... 12.5 g
- Bile Salts No. 3 ................................. 1.5 g
- Sodium Chloride ................................. 5.0 g
- Agar ................................................ 15.0 g
- Aniline Blue ...................................... 0.1 g

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

**Difco™ m FC Broth Base**

Consists of the same ingredients without the agar.

**Difco™ Rosolic Acid**

Rosolic Acid ................................................ 1 g/vial

Directions for Preparation from Dehydrated Product

**Difco™ m FC Agar**

1. Suspend 52 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. Add 10 mL of a 1% solution of Rosolic Acid in 0.2N NaOH. Continue heating for 1 minute. DO NOT AUTOCLAVE.
4. If necessary, adjust to pH 7.4 with 1N HCl.
5. Test samples of the finished product for performance using stable, typical control cultures.

**Difco™ m FC Broth Base**

1. Suspend 3.7 g of the powder in 100 mL of purified water.
2. Add 1 mL of a 1% solution of Rosolic Acid in 0.2N NaOH.
3. If necessary, adjust to pH 7.4 with 1N HCl.
4. Heat to boiling. DO NOT AUTOCLAVE.
5. Cool before dispensing.
6. Test samples of the finished product for performance using stable, typical control cultures.

**Difco™ Rosolic Acid**

Prepare a 1% solution, dissolving 1 g in 100 mL of 0.2N NaOH.

Procedure

**Difco™ m FC Agar**

1. Prepare the agar medium from the dehydrated base according to the label directions and with the addition of the Rosolic Acid solution.
2. Pour molten agar, previously cooled to 45-50°C into special tight-fitting plastic dishes and allow to harden.
3. Roll the membrane filter used to collect the water sample onto the surface of the agar, so as to avoid the formation of air bubbles between the filter and the agar surface.
4. Place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

**Difco™ m FC Broth**

1. Prepare the broth medium from the dehydrated base according to the label directions and with the addition of the Rosolic Acid solution.
2. Add 2 mL of the cooled broth to sterile absorbent pads in special tight-fitting plastic dishes.
3. Roll the membrane filter used to collect the water sample onto the moistened absorbent pad, so as to avoid the formation of air bubbles between the filter and the pad.
4. Place the dishes in plastic bags and incubate, by immersion, in a water bath at 44.5 ± 0.2°C for 24 ± 2 hours.

Expected Results

Colonies of fecal coliforms will be various shades of blue. Non-fecal coliforms are gray to cream-colored.

Limitation of the Procedure

A few non-fecal coliform colonies may be observed on m FC media due to the selective action of the elevated temperature and the addition of the Rosolic Acid. It may be useful to elevate the temperature to 45 ± 0.2°C to eliminate *Klebsiella* strains from the fecal coliform group.

References


Availability

**Difco™ m FC Agar**

- AOAC Cat. No. 267710 Dehydrated – 100 g
- CCAM Cat. No. 267720 Dehydrated – 500 g
- EPA SMWW
- Difco™ m FC Broth Base

- EPA SMWW
- SMWW
- Difco™ Rosolic Acid

Cat. No. 232281 Vial – 6 × 1 g