**KF Streptococcus Broth**

**Intended Use**

KF Streptococcus Broth is used for isolating fecal streptococci.

**Summary and Explanation**

Kenner et al. developed KF (Kenner Fecal) Streptococcal Broth for the detection and enumeration of enterococci in waters.\(^1\)\(^2\) They found that this formulation was superior to other liquid media in the recovery of enterococci in Most Probable Number (MPN) test systems. The medium is not specific for presumptive identification of group D streptococci. Other tests are required.\(^2\)\(^4\)

**Principles of the Procedure**

Peptone provides a source of nitrogen, amino acids and carbon. Yeast extract is a source of trace elements, vitamins and amino acids. Maltose and lactose are the fermentable carbohydrates and carbon sources. Sodium azide is the selective agent. Brom cresol purple is the indicator dye.

The addition of 1% TTC (2,3,5-Triphenyl Tetrazolium Chloride), in the membrane filter procedure, causes the enterococci to have a deep red color as a result of tetrazolium reduction to an acid azo dye.

### User Quality Control

**Identity Specifications**

**Difco™ KF Streptococcus Broth**

- **Dehydrated Appearance:** Light greenish-beige, free-flowing, homogeneous.
- **Solution:** 5.64% solution, soluble in purified water upon boiling. Solution is reddish to light purple, clear to very slightly opalescent.
- **Prepared Appearance:** Purple, clear to very slightly opalescent.
- **Reaction of 5.64% Solution at 25°C:** pH 7.2 ± 0.2

**Cultural Response**

**Difco™ KF Streptococcus Broth**

Prepare the medium per label directions. Supplement with TTC Solution 1%. Using the membrane filter technique, inoculate and incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 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>Enterobacter aerogenes</td>
<td>13048</td>
<td>3 x 10^2-10^3</td>
<td>Inhibition</td>
<td>–</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>19433</td>
<td>30-200</td>
<td>Good</td>
<td>Red</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>29212</td>
<td>30-200</td>
<td>Good</td>
<td>Red</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>3 x 10^2-10^3</td>
<td>Inhibition</td>
<td>–</td>
</tr>
</tbody>
</table>
**Formula**

**Difco™ KF Streptococcus Broth**

*Approximate Formula* Per Liter

- Proteose Peptone No. 3: 10.0 g
- Yeast Extract: 10.0 g
- Sodium Chloride: 5.0 g
- Sodium Glycerophosphate: 10.0 g
- Maltose: 20.0 g
- Lactose: 1.0 g
- Sodium Azide: 0.4 g
- Bromcresol Purple: 15.0 mg

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

**Directions for Preparation from Dehydrated Product**

**MPN Procedure**

1. For an inoculum of 1 mL or less, suspend 56.4 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. For an inoculum of 10 mL, suspend 84.6 g of the powder in 1 L of purified water. Mix thoroughly.
4. Dispense 10 mL amounts into culture tubes.
5. Autoclave at 121°C for 10 minutes.
6. Test samples of the finished product for performance using stable, typical control cultures.

**Membrane Filter Procedure**

1. Suspend 56.4 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. Dispense in 100 mL amounts into flasks and autoclave at 121°C for 10 minutes.
4. Cool to 60°C and add 1 mL TTC Solution 1% per 100 mL of medium.
5. Test samples of the finished product for performance using stable, typical control cultures.

**Procedure**

**MPN Procedure**

1. Inoculate tubes of the KF Streptococcus Broth with the appropriate amount of inoculum.
2. Incubate tubes at 35 ± 1°C, with loosened caps, for 46-48 hours.

**Membrane Filter Procedure**

1. Place a sterile absorbent pad in each sterile Petri dish.
2. Saturate the pads with the sterile medium containing TTC.
3. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
4. Incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 hours.

**Expected Results**

**MPN Procedure**

MPN tubes positive for enterococci are turbid with growth that appears yellow in color and does not produce foaming. When foaming occurs, confirmation for enterococci should be made by Gram staining.

**Membrane Filter Procedure**

All red or pink colonies visible with 15× magnification are counted as enterococci colonies.

**Limitations of the Procedure**

1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
2. Overheating may lower the pH, resulting in a decrease in productivity of the medium.

**References**


**Availability**

**Difco™ KF Streptococcus Broth**

Cat. No. 212226 Dehydrated – 500 g

**Difco™ TTC Solution 1%**

Cat. No. 231121 Tube – 30 mL
264310 Bottle – 25 g