# KF Streptococcus Agar TTC Solution 1%

## Intended Use

KF Streptococcus Agar is used with TTC Solution 1% in isolating and enumerating fecal streptococci.

## Summary and Explanation

Kenner et al. developed KF (Kenner Fecal) Streptococcal Agar with TTC for use in detecting streptococci in surface waters by direct plating or by the membrane filtration method.[1] These investigators compared the performance of their formulation to other media used for enumerating fecal streptococci and achieved greater recoveries with KF Streptococcal Agar. The medium is recommended for use in determining counts of fecal streptococci in water.

TTC (2, 3, 5-Triphenyl Tetrazolium Chloride) in aqueous 1% solution may be added to various agar and fluid media to lend color to cells and to colonies on agar or on membranes, thereby facilitating the detection of growth.

## 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 fermentable carbohydrates and carbon sources. Sodium azide is a selective agent. Bromcresol purple is an indicator dye.

## User Quality Control

### *Identity Specifications*

**Difco™ KF Streptococcus Agar**

| | |
|---|---|
| Dehydrated Appearance: | Light greenish-beige, free-flowing, homogeneous. |
| Solution: | 7.64% solution, soluble in purified water upon boiling. Solution is light purple, very slightly to slightly opalescent. |
| Prepared Appearance: | Light purple, very slightly to slightly opalescent. |
| Reaction of 7.64% Solution at 25°C: | pH 7.2 ± 0.2 |

**Difco™ TTC (powder)**

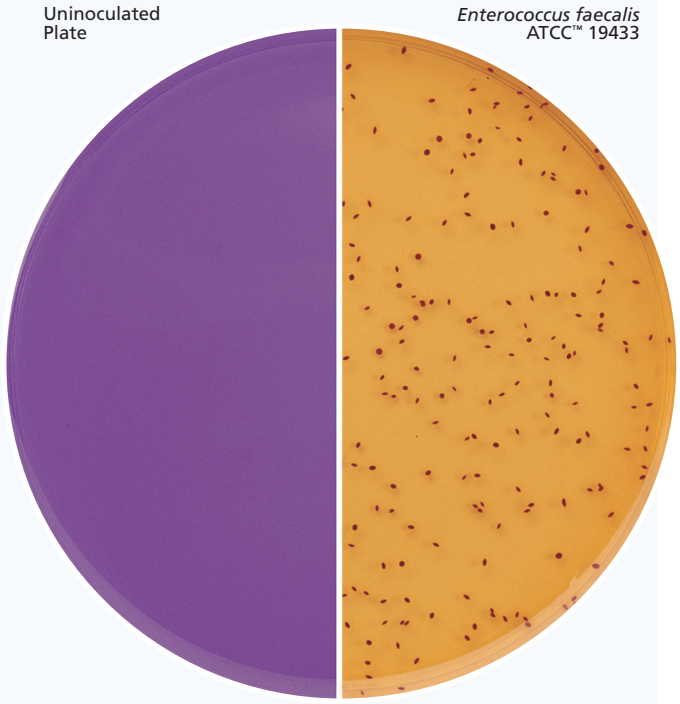
| | |
|---|---|
| Appearance: | White to slightly yellow, free-flowing, homogeneous. |
| Solution: | 1% solution, soluble in purified water. Solution is colorless to very slight amber, clear. |

### *Cultural Response*

**Difco™ KF Streptococcus Agar**

Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 46-48 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY | COLONY COLOR |
|---|---|---|---|---|
| *Enterobacter aerogenes* | 13048 | $3\times10^2$-$10^3$ | Marked to complete inhibition | – |
| *Enterococcus faecalis* | 19433 | 30-300 | Good | Red to pink centers |
| *Enterococcus faecalis* | 29212 | 30-300 | Good | Red to pink centers |
| *Escherichia coli* | 25922 | $3\times10^2$-$10^3$ | Marked to complete inhibition | – |



TTC is used as a redox indicator in culture media. It is colorless in the oxidized form and is reduced to formazan, an insoluble red pigment, by actively growing microbial cells. The reduction of TCC is irreversible; it is not reoxidized by air once it is reduced to the red formazan.[2]

In this medium, the addition of 1% triphenyltetrazolium chloride (TTC) causes enterococci to develop a deep red color.

## Formula

### Difco™ KF Streptococcus Agar

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 |
| Agar | 20.0 | g |

### TTC Solution 1%

Formula Per 100 mL

| | | |
|---|---|---|
| 2, 3, 5-Triphenyltetratolium Chloride | 1.0 | g |

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

## Directions for Preparation from Dehydrated Product

1. Suspend 76.4 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.
2. Heat an additional 5 minutes. Avoid overheating which could decrease the productivity of the medium. DO NOT AUTOCLAVE.
3. Aseptically add 10 mL TTC Solution 1% to the medium cooled to 50°C. Mix well.
4. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

### Pour Plate Technique

1. Prepare appropriate dilutions of the test material.
2. Place the selected volume of sample in a Petri dish.
3. Pour 15 mL of the prepared medium at 45-50°C into each plate.
4. Thoroughly mix the medium and sample to uniformly disperse the organisms.
5. Allow the agar to solidify.
6. Incubate plates in the inverted position at 35 ± 2°C for 46-48 hours.

### Membrane Filter Procedure

1. Filter a suitable volume of sample through a sterile membrane.
2. Place the inoculated membrane filter on the solidified agar in the Petri dish, inoculum side up.
3. Incubate the plates, inverted, at 35 ± 2°C for 46-48 hours.

## Expected Results

Enterococci will appear as red or pink colonies. The use of a stereoscopic microscope with 15× magnification can aid in counting colonies.

## Limitations of the Procedure

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

## References


1. Kenner, Clark and Kabler. 1961. Appl. Microbiol. 9:15.
2. Kelly and Fulton. 1953. Am. J. Clin. Pathol. 23:512.


## Availability

### Difco™ KF Streptococcus Agar

Cat. No. 249610 Dehydrated – 500 g

### Difco™ TTC Solution 1%

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

**Store at 2-8°C.*