

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. Bordner and Winter. 1978. Microbiological methods for monitoring the environment, water and wastes. Environmental Protection Agency, Cincinnati, Ohio.
3. Kelly and Fulton. 1953. Am. J. Clin. Pathol. 23:512.

Availability

Difco™ KF Streptococcus Agar

COMPF EPA

Cat. No. 249610 Dehydrated – 500 g

Mexico

Cat. No. 222050 Prepared Plates – Pkg. of 10*

Difco™ TTC Solution 1%

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

*Store at 2-8°C.

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. Bromcresol 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.

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.

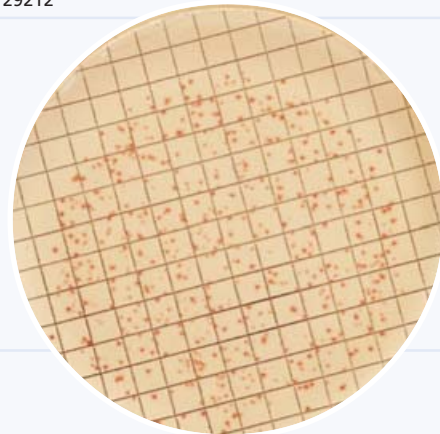
User Quality Control

Enterococcus faecalis
ATCC™ 29212

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.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterobacter aerogenes</i>	13048	3 × 10 ² -10 ³	Inhibition	–
<i>Enterococcus faecalis</i>	19433	30-200	Good	Red
<i>Enterococcus faecalis</i>	29212	30-200	Good	Red
<i>Escherichia coli</i>	25922	3 × 10 ² -10 ³	Inhibition	–

Directions for Preparation from Dehydrated Product

MPN Procedure

- For an inoculum of 1 mL or less, suspend 56.4 g of the powder in 1 L of purified water. Mix thoroughly.
For an inoculum of 10 mL, suspend 84.6 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- For an inoculum of 1 mL or less, dispense 10 mL amounts into culture tubes.
For an inoculum of 10 mL, dispense 20 mL amounts into culture tubes.
- Autoclave at 121°C for 10 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Membrane Filter Procedure

- Suspend 56.4 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Dispense in 100 mL amounts into flasks and autoclave at 121°C for 10 minutes.
- Cool to 60°C and add 1 mL TTC Solution 1% per 100 mL of medium.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

MPN Procedure

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

Membrane Filter Procedure

- Place a sterile absorbent pad in each sterile Petri dish.
- Saturate the pads with the sterile medium containing TTC.
- Place an inoculated membrane filter, inoculated side up, on the saturated pad.
- 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

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

References

- Kenner, Clark and Kabler. 1960. Am. J. Public Health 50:1553.
- Kenner, Clark and Kabler. 1961. Appl. Microbiol. 9:15.
- MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
- Facklam and Moody. 1970. Appl. Microbiol. 20:245.

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

KL Virulence Agar KL Virulence Enrichment • KL Antitoxin Strips

Intended Use

KL (Klebs-Loeffler) Virulence Agar is used with KL Virulence Enrichment, Tellurite Solution 1% and KL Antitoxin Strips in differentiating virulent (toxigenic) from nonvirulent strains of *Corynebacterium diphtheriae*.

Summary and Explanation

Elek¹ was the first to describe the agar plate diffusion technique for demonstrating the *in vitro* toxigenicity (virulence) of *Corynebacterium diphtheriae*. King, Frobisher and Parsons² expanded on Elek's technique and, by using a carefully standardized medium, obtained results in agreement with animal inoculation tests. These authors demonstrated that Proteose Peptone possessed properties essential for toxin pro-

duction. Incorporating Proteose Peptone into the test medium assured consistent results. The authors used rabbit, sheep and horse serum as enrichments, finding human serum to be unsatisfactory. To overcome irregularities encountered in previous formulations, Hermann, Moore and Parsons³ refined the medium used for the *in vitro* KL Virulence Test, simplifying the basal medium and developing a nonserous enrichment. The medium and enrichment described by these authors have been standardized for use in the KL Virulence Test.

KL Virulence Agar and KL Virulence Enrichment are prepared according to the formulation of Hermann, Moore and Parsons.³