KF Streptococcal Agar

Cat #: MM-M-034

For the selective isolation and enumeration of fecal enterococci by direct culture or by membrane-filtration

Principles and uses:

KF Streptococcal Agar is a selective medium for the isolation and enumeration of fecal enterococci in water, foodstuffs and other materials, according to the formula developed by Kenner, Clark and Kabler.
It is used for the plate count of enterococci in water samples and for determining the presence of Enterococcus faecalis in milk and its derivatives, as well as in other foods. The isolation and enumeration of fecal enterococci is made according to APHA for the examination of water (1998) and foodstuffs (1992).
Peptone mixture provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Maltose and Lactose are the fermentable carbohydrates as carbon and energy sources. Sodium glycerophosphate is a buffering agent. Sodium azide is a selective agent that inhibits Gram-negative bacteria. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.
The addition of TTC 1% Supplement (2,3,5-tryphenyltetrazolium-chloride) allows fecal enterococci to develop a red color as the result of the reduction of tetrazolium to formazan, an insoluble red pigment, by actively growing microbial cells.

Formula per Litre:

Bacteriological agar	20g	Lactose	1g
Maltose	20g	Peptone mixture	10g
Sodium azide	0.4g	Sodium chloride	5g
Sodium glycerophosphate	10g	Yeast extract	10g

Preparation:

Suspend 76.4 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. AVOID OVERHEATING. DO NOT AUTOCLAVE. Cool to 45-50ºC and aseptically add two vials of TTC 1% Supplement (Cat. MMS-M-X030), previously reconstituted (each one) in 5ml of sterile distilled water. Homogenize gently and dispense into Petri dishes.

Instructions for use:

Pour plate method:
- Place selected dilution of sample in Petri Dish.
- Pour 15 ml of prepared medium at 45ºC into each plate.
- Thoroughly mix and allow agar to solidify.
- Incubate plates in inverted position at 35±2ºC for 46–48 hours.

Membrane filter technique:
- Filter suitable volume of sample through sterile membrane.
- Place membrane filter, inoculum side up, on solidified agar in Petri dish.
- Incubate inverted plates at 35±2 ºC for 46-48 hours.

The red or pink colonies are counted as fecal enterococci, while colonies with orange, yellow, white or other colors are not counted. The number of fecal enterococci is calculated per 100 ml of water.

Quality control:

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Clear amber with a pink tint	7.2 ± 0.2

Microbiological test:

Incubation conditions: (35±2ºC / 46-48h).

Microorganisms	Specification	Characteristic reaction
Enterobacter aerogenes ATCC 13048	Inhibited growth	
Enterococcus faecalis ATCC 19433	Good Growth	Red Colony
Escherichia coli ATCC 25922	Inhibited growth	
Enterococcus faecalis ATCC 29212	Good Growth	Red Colony

Storage:

Temperature: 2ºC - 25ºC

Bibliography:

Ramos Cordova, Mario. "Manual of Methods of Milk and Lactose Analysis". Edition of Author, Mexico, D. F., 1976.
Kenner, Clark and Kabler, Applied Microbiol. 9:15. 1961.
Donnelly C.W., R.E. Bracket, D.Doores, W.H. Lee, and J. Lovett. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.