Formula * in g/L

Proteose peptone	10.000
Yeast extract	10.000
Sodium chloride	5.000
Sodium glycerophosphate	10.000
Maltose	20.000
Lactose	1.000
Sodium azide	0.400
Bromocresol purple	0.015
Agar	20.000

Final pH 7,2 ±0,2 at 25 ºC

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

Directions

Suspend 76.4 g of powder in 1 L of distilled water and bring to the boil stirring constantly. If it is to be used immediately, sterilisation is not necessary. If sterilization is necessary, sterilize in the autoclave in small volumes, at 121°C for 10 minutes maximum. Let agar cool to 50°C and add 10 mL/L of TTC Sterile Solution 1% (Art. No. DSHB3074). Homogenize well and distribute into Petri dishes.
Note: A non-homogeneous appearance is normal, and does not affect the medium´s quality and efficacy.

Description

Kenner, Clark and Kabler (1960, 1961) discovered that KF medium was excellent for detecting enterococci in polluted water. Carbohydrates in this medium (lactose and maltose) are utilised by most of enterococci, producing a big amount of acid and causing the indicator to turn from violet to yellow. Streptococci that do not belong the D group may also grow in the medium, but they do not produce enough acid to change the colour of the indicator. Other microorganisms are strongly inhibited by sodium azide. Enterococci reduce TTC to formazan and so their colonies are red in colour.

Technique

If the sample is suspected of being highly contaminated, prepare ten-fold serial dilutions bank and inoculate the surface with 0,1 mL of sample using a Drigalsky loop (spread plate method) or, if desired, inoculate the medium with 1 mL of sample using the pour plate method. Incubation should be carried out at 37°C for a 48 hours period.
After incubation, readings are performed by observing whether the indicator has turned from violet to yellow, and whether colonies are pink or red in colour.
It is very important to maintain the pH of the medium over 7,0, or otherwise, false results may occur. Sterilization for longer than the specified period could result in darkening of the sugar and thereby resulting in a decrease in the pH.

Quality control

Incubation temperature: 37ºC ± 1 **Incubation time:** 48 h ± 3

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) /10^4-10^6 CFU (Selectivity). Spiral Plate Method (or Membran Filter Method).

Microorganism	Growth	Remarks
Pseudomonas aeruginosa ATCC® 27853	Total inhibition	Selectivity
Escherichia coli ATCC® 25922	Total inhibition	Selectivity
Enterococcus faecalis ATCC® 29212	Productivity > 0.50	Dark red colonies.Yellowish medium
Enterococcus faecalis ATCC® 19433	Productivity > 0.50	Dark red colonies.Yellowish medium

References

· CLESCERI, L.S., A.E. GREENBERG & A.D. EATON (1998) Standard Methods for the Examination of Water and Wastewater 20th ed. APHA, Washington.
· DOWNES, F.P. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
· KENNER, B.A., CLARK, H.F. & KABLER, P.W. (1960) Fecal Streptococci I. Cultivation and Enumeration of Streptococci in Surface Waters. Appl. Microbiol. 9:15.
· KENNER, B.A., CLARK, H.F. & KABLER, P.W. (1961) Fecal Streptococci II. Quantification of Streptococci in faeces. Am. Inst. Publ. Health, 50:1553.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).