17185 M Enterococcus Agar, modified
(Membrane filter Enterococcus Agar, modified)

Selective medium for enumeration and detection of enterococci acc. to Slanetz and Bartley (1957) in water and sewage with the membrane-filter technique and in the direct application procedure in food acc. to Burkwall and Hartman (1964).

Composition:

Ingredients	Grams/Litre
Pancreatic digest of gelatin	10.0
Yeast extract	30.0
Sodium chloride	15.0
Esculin	1.0
Sodium azide	0.15
Cycloheximide	0.05
Nalidixic acid	0.25
Agar	15.0
Final pH 7.1 ± 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:
Suspend 71.4 g in 1 litre distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 45°C and add aseptically 15 ml of sterile 1% TTC Solution (Cat. No. 17779). Mix well and pour into sterile petri plates. Warning: Cycloheximide is very toxic. Avoid skin contact or aerosol formation and inhalation!

Principle and Interpretation:
"M Enterococcus Agar Base, modified" was developed for the enumeration and identification of Enterococci in the water of swimming pools, according to USEPA. Cabelli et al established the correlations between enterococcal densities and gastroentitis associated with swimming in recreational waters. This medium is also useful for the detection and quantification of Enterococci from all kinds of water.
Pancreatic digest of gelatin and yeast extract act as a source of carbon, nitrogen, minerals, vitamins and other essential nutrients for growth. Sodium chloride maintains isotonic conditions of the medium besides the provision of essential ions to a variety of organisms. Sodium azide, Cycloheximide and Nalidixic acid inhibit many species of bacteria and fungi and thus makes the medium selective. Esculin is hydrolyzed by bacterial enzyme to esculetin and dextrose. TTC is reduced by Enterococci to insoluble formazan inside the bacterial cells which gives red colouration to colonies.
For the membrane filter procedure, two culture media are established for the enumeration and identification of Enterococci where "M Enterococcus Agar, modified" serves as a selective medium while Esculin Iron Agar (Esculin 1g/l, Ferric ammonium citrate 0.5g/l, Agar 15g/l) confirms the identification of colonies on the basis of ability of organisms to hydrolyze esculin. Initially the membrane filter that has been used for filtering the water is placed on the "M Enterococcus Agar, modified" plate and incubated at 41°C for 48 hours. After incubation the filter is transferred to the Esculin Iron Agar plate and incubate for further 20 minutes at 41°C.

Cultural characteristics after 48 hours at 40-42°C.

Organisms (ATCC)	Growth	Color of Colony	Esculin hydrolysis
Entreococcus faecalis (11700)	+++	pink to dark red	+ (black to brown colonies on Esculin Iron Agar)
Escherichia coli (25922)	-	-	-

References:

1. United States Environmental Protection Agency, EPA - 600/h-85/076. USEPA, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio (1985)
2. Cabelli, Dufour, Levin, et al, Am. J. Public Health 69, 690 (1979)
3. A. E. Greenberg, R. R. Trussell and L.S. Clesceri (Eds.), Standard Methods for the Examination of Water and Wastewater, 16th ed., APHA, Washington, D.C. (1985)
4. Bordner, Winter and Scarpino (Eds.), EPA - 600/8-78-017 USEPA, Office of Research and Development, Environmental Monitoring and Support Laboratory Gincinnati, Ohio (1978)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.