TM 2185 - M-ENTEROCOCCUS AGAR BASE, MODIFIED

INTENDED USE
For cultivation and enumeration of Enterococci from water samples using membrane filter technique.

PRODUCT SUMMARY AND EXPLANATION
According to the USEPA, M-Enterococcus Agar Base, modified was created for the enumeration and identification of Enterococci in sanitary quality recreational water. Cabelli et al. discovered a link between enterococcal density and gastroentitis linked with recreational swimming. This medium is also useful for the detection and quantitiation of *Enterococci* from potable, fresh, esturine, marine and shellfish growing waters.

COMPOSITION

Ingredients	Gms / Ltr
Pancreatic digest of casein	10.000
Sodium azide	0.150
Yeast extract	30.000
Sodium chloride	15.000
Esculin	1.000
Cycloheximide	0.050
Nalidixic acid	0.250
Agar	15.000

PRINCIPLE
Gelatin peptone and yeast extract provide the carbonaceous and nitrogenous nutrients, minerals, vitamins and other growth factors. Sodium chloride maintains isotonic conditions of the medium and essential ions to variety of organisms. Sodium azide, Cycloheximide and Nalidixic acid make medium selective by inhibiting large number of bacteria and fungi. Esculin is hydrolyzed by bacterial enzyme to esculetin and dextrose. *Enterococci* reduced TTC to insoluble formazan, a red colored complex inside the bacterial cell resulting in pink to red colored colonies.
In this membrane filter procedure, two culture media are used for the enumeration and identification of *Enterococci* that includes the M-Enterococcus Agar Base, Modified and Esculin Iron Agar. M-Enterococcus Agar, Modified act as a selective medium and Esculin Iron Agar used for identification of colonies which differentiate this organisms, on the basis of ability of organisms to hydrolyze esculin. The membrane filter used to filter the water is first placed on an M-Enterococcus Agar, Modified plate and incubated at 41°C for 48 hours before being moved to an Esculin Iron Agar plate and incubated for another 20 minutes at 41°C.
After incubation, count and record the colonies on membrane filters with 20 to 60 pink to red colonies on the underside of the membrane and black or reddish-brown precipitate. A magnifying glass and a fluorescent lamp can be used to count the visible colonies if necessary. For the final computation, apply the formula below.

$$\text{Enterococci/100ml} = \frac{\text{No. of Enterococcal colonies}}{\text{Volume of sample filtered (ml)}} \times 100$$

INSTRUCTION FOR USE
- Dissolve 71.45 grams in 1000 ml distilled water.
- Heat to boiling the medium to dissolve completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45°C and add aseptically 15 ml of sterile 1% TTC Solution.

- Mix well and pour into sterile Petri plates.

Warning: Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables. Cycloheximide is very toxic. Avoid skin contact or aerosol formation and inhalation.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Appearance of prepared medium : Yellow colored clear to slightly opalescent gel forms in Petri plates.

pH (at 25°C) : 7.1 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Color of the colony (on membrane filter)	Esculin hydrolysis	Incubation Temperature	Incubation Period
Escherichia coli	25922	>=10^3	Inhibited	0%	-	-	40-42°C	48 Hours
Enterococcus faecalis	29212	50-100	Luxuriant	>=70%	Pink to red(on the membrane filter)	Positive reaction, black to brown precipitate on the underside of membrane filter under individual colony	40-42°C	48 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

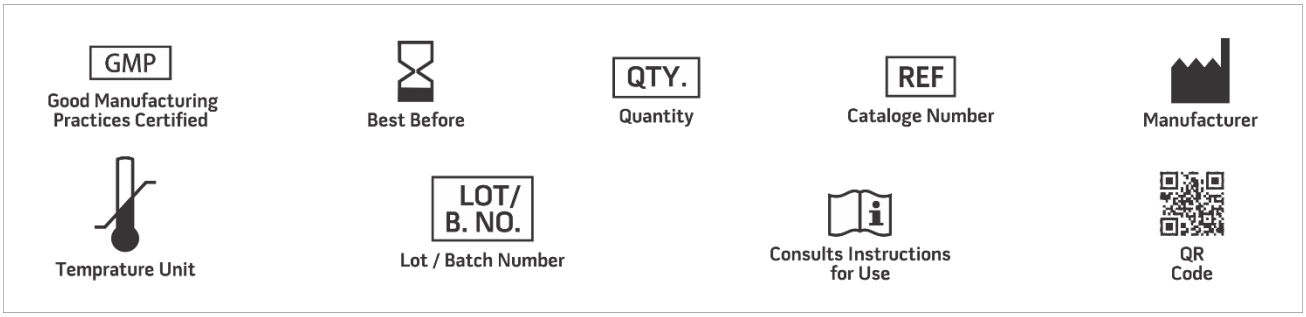
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. U. S. Environmental Protection Agency, 1997, EPA Method 1600: Membrane Filter test Methods for Enterococci in water,USEPA, EPA-821-R-97-004, Washington D. C.
2. Cabelli, Dufour, Levin, et al, 1979, Am. J. Public Health 69:690.
3. Greenberg A. W., Eaton A. D. and Clesceri L. S. (Eds.), 1998, Standard Methods for the Examination of Water and WasteWater, 20th ed., APHA, Washington DC
4. Bordner, Winter and Scarpino (Eds.), 1978, EPA - 600/8-78-017 USEPA, Office of Research and Development,Environmental Monitoring and Support Laboratory Cincinnati, Ohio.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williamsand Wilkins, Baltimore.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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