



M-Slanetz Enterococcus Broth Base

M1113

M-Slanetz Enterococcus Broth Base is used for isolation and detection of Enterococci using membrane filter technique.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	25.000
Peptone	15.000
Yeast extract	10.000
Dextrose	2.000
Sucrose	100.000
Dipotassium phosphate	4.000
Sodium azide	0.400
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 156.4 grams in 1000 ml distilled water. Heat, if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 1 vial of 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC, FD057). Mix well before dispensing.

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.

Principle And Interpretation

The membrane filter technique is employed when relatively large sample volumes are to be analyzed. This technique is highly reproducible and more rapid. Membrane filters are aseptically placed on sterile cotton absorbent filter pads saturated with the appropriate media. The Enterococci portion of the faecal *Streptococcus* group is a valuable bacterial indicator for determining the extent of faecal contamination of recreational surface waters (1). M-Slanetz Enterococcus Broth Base is formulated according to Slanetz and Bartley (2) for the isolation and detection of Enterococci using the membrane filter technique (3). This medium is a modification of M- Enterococcus Agar developed by Slanetz, Bent and Bartley (4).

Casein enzymic hydrolysate, peptone and yeast extract provide necessary nutrients like nitrogenous compounds and vitamin B complex. Dextrose and sucrose are the fermentable carbohydrate sources in the medium. Dipotassium phosphate helps in buffering the medium. Sodium azide inhibits the growth of most of the accompanying gram-negative microbial flora. Triphenyl Tetrazolium Chloride is reduced by Enterococci to formazan, a red coloured complex inside the bacterial cell resulting in the formation of red coloured colonies.

Saturate sterile absorbent cotton pads with M-Slanetz Enterococcus Broth Base. Aseptically place the membrane filters, through which water sample is passed, onto these saturated sterile absorbent cotton pads. Incubate at 35-37°C for 40-48 hours. Enterococci will form red coloured colonies on the surface of filter membranes. Refer appropriate references for standard procedures (1).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured clear solution without any precipitate

Reaction

Reaction of 15.6% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

M1113: Cultural characteristics observed on membrane filter after an incubation at 35-37°C for 40-48 hours

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane filter)
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Cultural Response

<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited	
<i>Enterococcus faecalis</i> ATCC 50-100 29212		luxuriant	red- maroon

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Eaton A. D., Clesceri L. S. and Greenberg A. E., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
2. Slanetz L. W. and Bartley C. H., 1957, J. Bacteriol., 74: 591.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
4. Slanetz L. W., Bent D. and Bartley C. H., 1955, Public Health Rep.,70: 67.

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