Technical Information

M-Enterococcus Agar Base

Product Code: DM 2108

Application: - M-Enterococcus Agar Base is a selective medium used in membrane filtration procedures as well as a direct plating medium, for isolation and enumeration of Enterococci in water, sewage, food or other materials.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	15.000
Papaic digest of soyabean meal	5.000
Yeast extract	5.000
Dextrose	2.000
Dipotassium phosphate	4.000
Sodium azide	0.400
Triphenyl tetrazolium chloride	0.100
Agar	10.000
Final pH (25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Slanetz, Bent and Bartley formulated this media [1] for the enumeration of Enterococci by the membrane filter technique [2] They further modified it by the addition of Triphenyl Tetrazolium Chloride (TTC) and found that larger colonies and higher counts were obtained by placing membrane filters after filtration directly on the agar surface than on pads saturated with liquid medium. Burkwell and Hartman used polysorbate 80 (0.5 ml/l) and sodium carbonate (2 ml of a 10% aqueous solution per litre) to increase sensitivity of the media for direct plating of foods and increasing colony size [3]. As per standard methods, M-Enterococcus Agar is used for the detection of faecal Streptococcus and Enterococcus groups using the membrane filtration technique [4] from water, sewage, food and other material.

Casein enzymic hydrolysate and papaic digest of soyabean meal, yeast extract, dextrose act as source of carbon, nitrogen and other essential growth nutrients. Sodium azide inhibits gram-negative organisms. TTC serves as a rapid indicator of bacterial growth. TTC is reduced to insoluble formazan inside the bacterial cells, which gives red colouration to colonies.

Transfer the filter aseptically to agar medium, avoiding air bubbles beneath the membrane & Incubate the plates at 35°C for 48 hours. The medium can also be directly inoculated by streaking the specimen and incubating the plates at 35-37°C for 24-48 hours. After incubation, count all light and dark red colonies as Enterococci. For best result select the sample size to be filter to obtain 20-600 colonies after placing membrane filter on agar surface.

Methodology

Suspend 41.5 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. DO NOT OVERHEAT OR AUTOCLAVE. Add 0.5 ml polysorbate 80 and 2 ml of 10% aqueous solution of sodium carbonate, if desired. Dispense into 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.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder
Gelling
Firm, comparable with 1.0% Agar gel.
Colour and Clarity of prepared medium
Light pink coloured clear to slightly opalescent gel forms in Petri plates.
Reaction
Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.2±0.2
pH range 7.00-7.40
Cultural Response/ characteristices
DM 2107: Cultural characteristics observed after an incubation at 35-37°C for 24 - 48 hours.

Organism	Inoculum (CFU)	Growth	Colour of Colony (on Membrane filter)
Escherichia coli ATCC 25922	>=10^3	inhibited	
Enterococcus faecalis ATCC 29212	50-100	good-luxuriant	pink – dark red (maroon)

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^{o} in sealable plastic bags for 2-5 days.

Further Reading

1. Slanetz, Bent and Bartley, 1955, Publ. Health. Rep., 70:67.
2. Slanetz and Bartley, 1957, J. Bact., 74:591.
3. Burkwell and Hartman, 1964, Appl. Microbiol., 12:18.
4. Eaton A. D., Clesceri L. S. and Greenberg A. W., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington, D.C.

Disclaimer :

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