HiCrome™ M-TEC Agar

M1571

Intended Use

Recommended for differentiation and enumeration of thermotolerant *Escherichia coli* from water by membrane filter technique.

Composition**

Ingredients	g / L
Proteose peptone	5.000
Yeast extract	3.000
Lactose	10.000
Sodium chloride	7.500
Dipotassium hydrogen phosphate	3.300
Potassium dihydrogen phosphate	1.000
Sodium lauryl sulphate (SLS)	0.200
Sodium deoxycholate	0.100
Chromogen	0.500
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.6 gram in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

HiCrome™ M-TEC Agar is a chromogenic media used for detection and enumeration of thermo-tolerant *E.coli* (TEC)in water by membrane filtration developed by Dufour (1). The modified medium contains the chromogen, 5-bromo-6-chloro-3-indolyl-β-D-glucuronide that is cleaved by enzyme β-D-glucuronidase to yield glucuronic acid, produced by *E.coli* strains. This imparts a purple-magenta colour to the colonies of *E. coli* only. *E.coli* can be isolated from samples. Proteose peptone and yeast extract provides carbon, nitrogen substances, long chain amino acids, vitamins and essential nutrients. Lactose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate provide strong buffering system to control the pH in the presence of fermentative action. Sodium lauryl sulphate and sodium deoxycholate make the medium more selective by inhibiting gram positive bacteria. Membrane filter through which water sample has been passed is aseptically placed on the medium. The plates are then incubated at 44.5 ± 0.2°C for 22 - 24 hours. Following incubation *E.coli* will form purple to magenta coloured colonies on the membrane filters.

Type of specimen

Water samples

Specimen Collection and Handling:

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2.Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3.Further biochemical and serological test are necessary for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 4.56% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 44.5±0.2°C for 22-24 hours.

Organism	Inoculum (CFU)	Growth	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	good to luxuriant	purple/ magenta
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	
Proteus mirabilis ATCC 25933	50-100	good	colourless-light brown
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good	tan-light purple

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Dufour,Strickland and Cabelli, 1981, Appl. Environ. Microbiol. 41: 1152.
2. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

Revision : 07/ 2024