



Technical Data

HiCrome™ M-TEC HiCynth™ Broth

MCD1713

Intended Use:

Recommended by the U.S. Environmental Protection Agency (USEPA) for detection of thermotolerant *Escherichia coli* in water by the membrane filtration technique. It can also be used to isolate *E.coli* from clinical samples.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No. 3 #	5.000
HiCynth™ Peptone No. 5#	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

**Formula adjusted, standardized to suit performance parameters

- Chemically defined peptone

Directions

Suspend 30.6 grams in 1000 ml purified / 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 desired quantity (2-5 ml of broth) on sterile absorbent cotton pad for saturation. The medium should be used within 24 hours after rehydration.

Principle And Interpretation

HiCrome M-TEC HiCynth™ Broth is a chromogenic media used for detection and enumeration of thermotolerant *E.coli* (TEC) in water by membrane filtration (5). HiCrome™ M-TEC Broth is a modification of the M-TEC Agar developed by Dufour (2). 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. HiCrome M-TEC HiCynth™ Broth is prepared by completely replacing animal based peptones with chemically defined peptones to avoid BSE/TSE /GMO risks associated with animal peptones.

HiCynth™ Peptone No.3 and HiCynth™ Peptone No.5 provides carbon and 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.

Saturate a sterile cotton absorbent pad with about 2 ml of HiCrome™ M-TEC HiCynth™ Broth. Membrane filter through which water sample has been passed is aseptically placed on the saturated absorbent pad face upwards. This absorbent pad is 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

Clinical samples : Blood, urine, Water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. β -glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative. 2. Some species may show poor growth due to nutritional variations.

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

Colour and Clarity of prepared medium

Light amber coloured clear solution in tubes

Reaction

Reaction of 3.06% 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 44.3-44.7°C for 22-24 hours

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

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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.),2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Dufour, Strickland and Cabelli, 1981, Appl. Environ. Microbiol. 41: 1152.
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.
5. U.S.Environmental Protection Agency, 2002, Method 1603; Publication EPA-821-R-02-023.

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Disclaimer :

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