HiCrome™ M-TEC Agar / HiCrome M-TEC Broth

Recommended by the U.S. Environmental Protection Agency (USEPA) for differentiation and enumeration of thermotolerant *Escherichia coli* in water by the membrane filtration technique.

M1571/
M1713

Composition **

	M1571	M1713
Ingredients	Grams/Litre	Grams/Litre
Proteose peptone	5.00	5.00
Yeast extract	3.00	3.00
Lactose	10.00	10.00
Sodium chloride	7.50	7.50
Dipotassium phosphate	3.30	3.30
Potassium dihydrogen phosphate	1.00	1.00
Sodium lauryl sulphate (SLS)	0.20	0.20
Sodium deoxycholate	0.10	0.10
Chromogen	0.50	0.50
Agar	15.00	—

Final pH (at 25°C) 7.3 ± 0.2
** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 45.6 grams of M1571 or 30.6 grams of M1713 in 1000 ml 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 & pour into sterile Petri plates (M1571). In M1713 aseptically add desired quantity (2-5 ml broth) on sterile absorbent pad for saturation in a sterile Petri plate. The medium should be used within 24 hours after rehydration.

Principle and Interpretation

HiCrome™ M-TEC Agar/Broth are the chromogenic media used for detection and enumeration of thermotolerant *Escherichia coli* (TEC) in water by membrane filtration (2). HiCrome™ M-TEC Broth is a modification of the M-TEC Agar 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.

Proteose peptone and yeast extract provides nitrogenous carbonaceous compounds, amino acids and long chain peptides for the growth of microoganisms. Lactose is the fermentable carbohydrate. Sodium chloride maintains osmotic equilibrium. Potassium dihydrogen phosphate and dipotassium 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 2ml of HiCrome™ M-TEC Broth (M1713). Membrane filter through which water sample has been passed is aseptically placed on the saturated absorbent cotton 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

Water samples

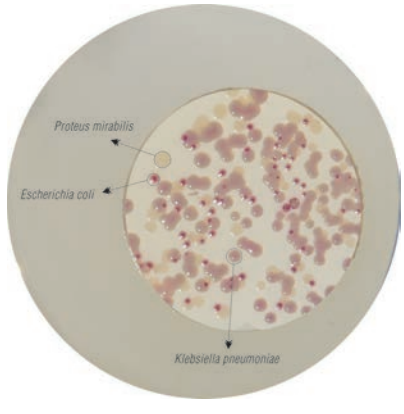
Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3).

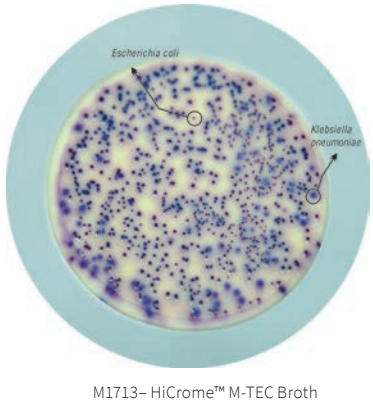
After use, contaminated materials must be sterilized by autoclaving before discarding.

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



M1571 – HiCrome™ M-TEC Agar



M1713– HiCrome™ M-TEC Broth

HiCrome™ M-TEC Agar / HiCrome M-TEC Broth

Recommended by the U.S. Environmental Protection Agency (USEPA) for differentiation and enumeration of thermotolerant *Escherichia coli* in water by the membrane filtration technique.

M1571/
M1713

Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Overgrowth of non-coliform organisms may interfere with the total coliform organisms.

Performance and Evaluation

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

Quality Control

Appearance of Powder : Cream to yellow homogeneous free flowing powder

Gelling : Firm, comparable with 1.5% Agar gel of M1571

Colour and Clarity of prepared medium : Light amber coloured, clear to slightly opalescent gel forms in Petri plates (M1571) / clear solution in tubes (M1713).

Reaction : Reaction of 4.56% w/v aqueous solution of M1571 and 3.06% w/v of M1713 at 25°C. pH : 7.3 ± 0.2 .

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

Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of Colony
Escherichia coli (25922) (00013*)	50-100	good to luxuriant	purple / magenta
Proteus mirabilis (25933)	50-100	good	colouless-light brown
Klebsiella pneumoniae (13883) (00097*)	50-100	good	colourless-tan
Enterococcus faecalis (29212) (00087*)	≥10^3	inhibited	—

Key : * : corresponding WDCM Numbers

Storage and Shelf-life

Store between 2-8°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 inorder 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. Use before expiry date on the label.

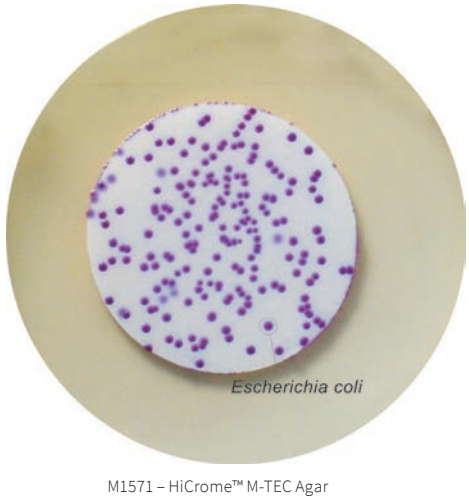
Product performance is best if used within stated expiry period.

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 (4, 5).

References

1. Dufour, Strickland and Cabelli, 1981, Appl. Environ. Microbiol. 41: 1152.
2. U.S. Environmental Protection Agency, 2002, Method 1603; Publication EPA-821-R-02-023.
3. 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
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
5. 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



M1571 – HiCrome™ M-TEC Agar