

For the detection of *E. coli* and thermotolerant coliforms

HiCrome™ Coliform Agar Modified

Recommended for the simultaneous detection of *Escherichia coli* and thermotolerant coliforms in water, milk, dairy products and other food samples.

M1832

Composition **

Ingredients	Grams/Litre
Peptone, special	8.000
Sodium chloride	1.000
Yeast extract	3.000
Potassium dihydrogen phosphate	0.200
Dipotassium phosphate	0.600
Bile salts	0.800
Magnesium sulphate	0.200
Chromogenic mixture	0.200
Agar	10.000

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 24 grams 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 and pour into sterile Petri plates.

Principle and Interpretation

HiCrome™ Coliform Agar Modified is a selective medium recommended for the simultaneous detection of *E. coli* and thermotolerant coliforms in water and food samples (4). Peptone special and yeast extract provide the necessary nitrogen compound, carbon, vitamins and also some trace ingredients required for the growth of organisms. The phosphates buffer the medium well. Magnesium sulphate helps colour development. Bile salts inhibits gram-positive organisms. Sodium chloride maintains osmotic balance.

The chromogenic mixture contains two chromogenic substrates, which enables the detection of two specific enzymes, β -galactosidase and β -glucuronidase. β -galactosidase produced by coliforms cleaves one chromogen, resulting in the pink colouration of coliform colonies. The enzyme β -glucuronidase produced by *E. coli*, cleaves X-glucuronide. *E. coli* forms dark blue to violet coloured colonies due to cleavage of both the chromogens (1, 2, 3). *E. coli* strains that are β -glucuronidase negative (serotype O157:H7) produce pink coloured colonies. Other gram negative bacteria able to grow at (44±0.5)°C produce white or colourless colonies.

Transfer 1 ml of product to analyse and its tenfold dilutions to sterile Petri plates. Pour 12 ml of medium, mix well and allow to solidify. Overlay with 4 ml of medium, allow to solidify and incubate at 43-45°C for 18-24 hours.

Type of specimen

Water, Food and Dairy samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (6, 7, 8).

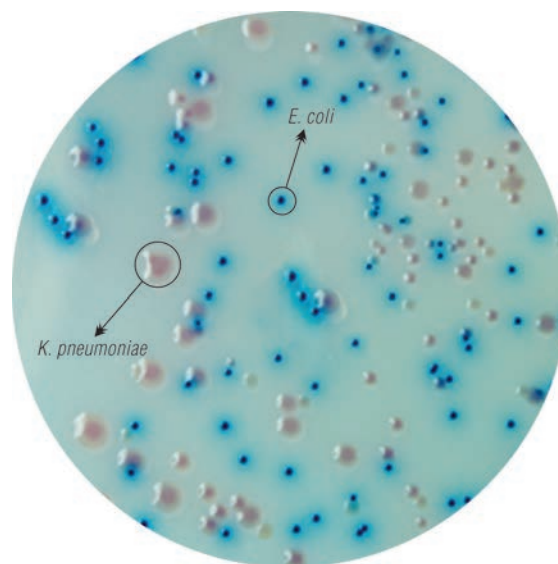
After use, contaminated materials must be sterilized by autoclaving before discarding.

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. 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 requirements.



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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** : Light yellow to beige homogeneous free flowing powder
- Gelling** : Firm, comparable with 1.0% Agar gel.
- Colour and Clarity of prepared medium** : Light yellow to slightly opalescent gel forms in Petri plates
- Reaction** : Reaction of 2.4% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2
- Cultural Response** : Cultural characteristics observed after an incubation at 43-45°C for 24 hours (48 hours if necessary).

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Escherichia coli</i> (10536)	50-100	good - luxuriant	≥50%	dark blue/violet
<i>Escherichia coli</i> (25922) (00013*)	50-100	good - luxuriant	≥50%	dark blue/violet
<i>Enterobacter cloacae</i> (23355) (00082*)	50-100	good - luxuriant	≥50%	pink
<i>Klebsiella pneumoniae</i> (13883) (00097*)	50-100	good - luxuriant	≥50%	light pink
<i>Enterococcus faecalis</i> (29212) (00087*)	≥10 ³	inhibited	0%	-
<i>Staphylococcus aureus</i> sub-sp aureus (25923) (00034*)	≥10 ³	inhibited	0%	-

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 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. 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 (9, 10).

References

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2. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
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7. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
10. 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

Ready Prepared Media			
Code	Product Name	Usage	Packing
Category : 90 mm Ready prepared Plates			
MP1832	HiCrome™ Coliform Agar Plate, Modified	it is a selective agar recommended for the simultaneous detection of <i>Escherichia coli</i> and total coliforms in water and food samples.	20 plts 50 plts