HiCrome™ E. coli Agar

Recommended for the detection and enumeration of *Escherichia coli* in foods and water without further confirmation on membrane filter or by indole reagent.

M1295/
M1295I

Composition **

	M1295	M1295I
Ingredients	Grams/Litre	Grams/Litre
Tryptone	14.00	20.00
Peptone, special	5.00	—
Bile salts mixture	1.50	1.50
Disodium hydrogen phosphate	1.00	—
Sodium dihydrogen phosphate	0.60	—
Sodium chloride	2.40	—
X-Glucuronide	0.075	0.075
Agar	12.00	15.00

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.57 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™ *E. coli* Agar is based on Tryptone Bile Agar to detect *Escherichia coli* in foods (1), where recovery of *E. coli* is faster, more reliable and accurate. Most of the *E. coli* strains can be differentiated from other coliforms by the presence of enzyme glucuronidase, which is highly specific for *E. coli* (2). Glucuronidase test is used increasingly for detection of *E. coli* in water and food microbiology as *E. coli* is an important indicator of fecal contamination in samples from the food processing and water purification plants. Other *Escherichia* spp. do not produce this enzyme (4). The chromogenic agent X-glucuronide used in this medium helps to detect glucuronidase activity of *E. coli*. *E. coli* cells absorb X-glucuronide and the intracellular glucuronidase enzyme splits the bond between the chromophore and the glucuronide. The released chromophore gives bluish green colouration to the *E. coli* colonies. Formulation of M1295I is in accordance with ISO (3).

Tryptone and peptone special provide the nitrogenous compounds, carbon, amino acids, vitamins and other essential growth nutrients to the organisms. Bile salts mixture inhibits gram-positive organisms. Sodium chloride and phosphates maintain osmotic balance and buffering action respectively.

The surface of the plated medium is dried before use. Dilute food samples 1:5 or 1:10 with 0.1% (w/v) sterile Peptone Water (M028) and homogenize in a blender or a stomacher. Pipette 0.5 ml or 1.0 ml of the homogenized food sample on to the plate and spread with sterile glass spreader. Incubate the plates at 30°C for 4 hours and then at 44°C for 18 hours.

Type of specimen

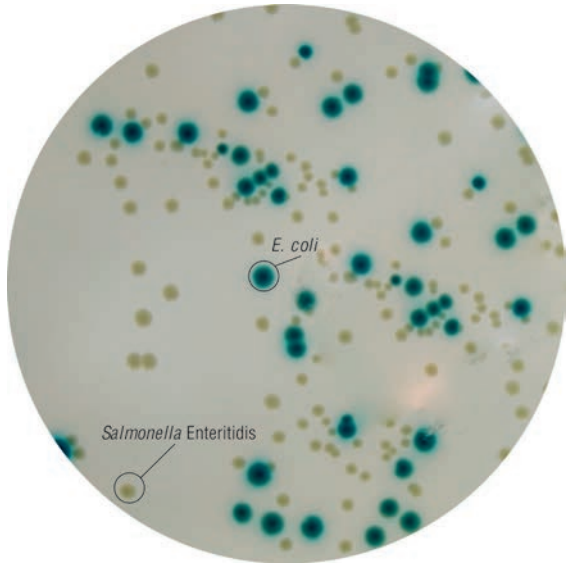
Water samples ; Food samples

Specimen Collection and Handling

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

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

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



M1295 – HiCrome™ *E. coli* Agar

HiCromeVeg™ Single Streak Rapid Differentiation Series *Freedom from BSE / TSE worries*

HiCrome™ ***E. coli*** Agar (M1295) is also available as HiCrome™ ***E. coli*** HiVeg™ Agar (MV1295) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

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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. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative hence *E.coli* species may show pink to red colonies.
2. Certain species of *Shigella* and *Salmonella* are ß-glucuronidase positive, hence they appear light blue to turquoise colonies.

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 coloured, homogeneous, free flowing powder.

Gelling : Firm, comparable with 1.2% Agar gel of M1295 or 1.5% Agar gel of M1295I .

Colour and Clarity of prepared medium : Light yellow coloured, clear to slightly opalescent gel forms in Petri plates.

Reaction : Reaction of 3.66% w/v aqueous solution at 25°C. pH:7.2 ± 0.2.

Cultural Response : Cultural characteristics observed after an incubation at 44°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
Escherichia coli (25922) (00013*)	50-100	luxuriant	≥50%	bluish green
Salmonella Enteritidis (13076) (00030*)	50-100	luxuriant	≥50%	colourless
Staphylococcus aureus subsp aureus (25923) (00034*)	$\geq 10^3$	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 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 (7, 8).

References

1. Anderson J. M. and Baird-Parker A. C., 1975, J. Appl. Bacteriol., 39:111.
2. Hansen W. and Yourassawsky E., 1984, J. Clin. Microbiol., 20:1177.
3. International Standard ISO 166492:1999. Microbiology of food and animal feeding stuff - horizontal method for the enumeration of presumptive *Escherichia coli*; Part 2: Colony count technique at 44°C using 5-bromo-4-chloro-3indolyl-β-D-glucornic acid.
4. Rice, E.W., Allen, M.J., Brenner, D.J., Edberg, S.C., 1991. Appl. Environ. Microbiol. 57, 592–593.
5. 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.
6. 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.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8. 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			
MP1295	HiCrome™ E.coli Agar Plate	for the detection and enumeration of *Escherichia coli* in foods without further confirmation on membrane filtration or by indole reagent	20 plts 50 plts
Category : HiTouch™ FlexiPlates			
FL002	HiTouch™ E.coli/Coliform Count Flexi Plate™	for enumeration (count) of all coliforms along with differential count of *E.coli*	50 plts