

HiCrome™ E. coli HiVeg™ Agar

MV1295

Intended Use:

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

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	14.000
HiVeg™ special peptone	5.000
Synthetic detergent	1.500
Disodium hydrogen phosphate	1.000
Sodium dihydrogen phosphate	0.600
Sodium chloride	2.400
X-Glucuronide	0.075
Agar	12.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 36.57 grams 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™ 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).

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. This medium is recommended for isolation of *E.coli* from water, food and clinical samples. This medium is prepared by completely replacing animal based peptones with vegetable peptones to avoid BSE /TSE risks associated with animal peptones. HiVeg™ hydrolysate and HiVeg™ special peptone special provides carbon, nitrogen compounds, long chain amino acids, vitamins and other essential growth nutrients to the organisms. Synthetic detergent 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.(3)

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (4).

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. Certain species of *Salmonella* are β -glucuronidase positive.
3. 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

Gelling

Firm, comparable with 1.2% Agar gel.

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

pH

7.00-7.40

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	$\geq 10^4$	inhibited	0%	
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	$\geq 50\%$	bluish green
<i>Salmonella</i> Enteritidis ATCC13076 (00030*)	50-100	luxuriant	$\geq 50\%$	colourless

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 (5,6).

Reference

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. Salfinger Y., and Tortorello M.L.,2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
4. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
6. 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.

Disclaimer :

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