

For identification and differentiation of *E. coli* and Total coliforms

HiCrome™ ECC Agar

Recommended as a differential medium for presumptive identification of *Escherichia coli* and other coliforms in food and environmental samples.

M1293

Composition **

Ingredients	Grams/Litre
Peptone, special	5.00
Yeast extract	3.00
Lactose	2.50
Disodium hydrogen phosphate	3.50
Potassium dihydrogen phosphate	1.50
Sodium chloride	5.00
Chromogenic mixture	20.30
Neutral red	0.03
Agar	15.00

Final pH (at 25°C) 6.8 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 55.83 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

Escherichia coli, a member of the family *Enterobacteriaceae* is a part of normal flora of the intestinal tract of humans and a variety of animals. Although most of *E. coli* does not cause gastrointestinal illnesses, certain groups of *E. coli* can cause life-threatening diarrhoea and severe sequelae or disability (1). HiCrome™ ECC Agar is a differential medium recommended for the presumptive identification of *E. coli* and other coliforms in food and environmental samples (2). The medium contains two chromogens. One of the chromogen is cleaved by the enzyme β -glucuronidase produced by *E. coli* to give blue to purple coloured colonies whereas the other chromogen is cleaved by the enzyme galactosidase, produced by majority of coliforms, resulting in the formation of rose-pink coloured colonies (3, 4).

Peptone special, yeast extract provide nitrogenous substances, carbonaceous compounds, long chain amino acids, vitamin B complex and other essential growth nutrients. Lactose is the fermentable carbohydrate, which aids in detecting lactose fermenters with neutral red as an indicator. Disodium hydrogen phosphate and potassium dihydrogen phosphate buffers the medium well. Sodium chloride maintains the osmotic equilibrium. Dry the surface of plate medium

before use.

Dilute the food sample 1: 5 or 1: 10 with 0.1% sterile Peptone Water (M028) and homogenize in a blender or a stomacher. Spread 0.5 ml or 1.0 ml of the homogenate over the agar surface with a sterile glass spreader and incubate the plates at 35-37°C for 18-24 hours. Count the blue/purple colonies and multiply with the dilution factor. The number of *E. coli* is reported per gram of food. The medium should be used only for in-vitro diagnostic purpose. Wear mask while handling the dehydrated product and avoid contact with eyes.

Type of specimen

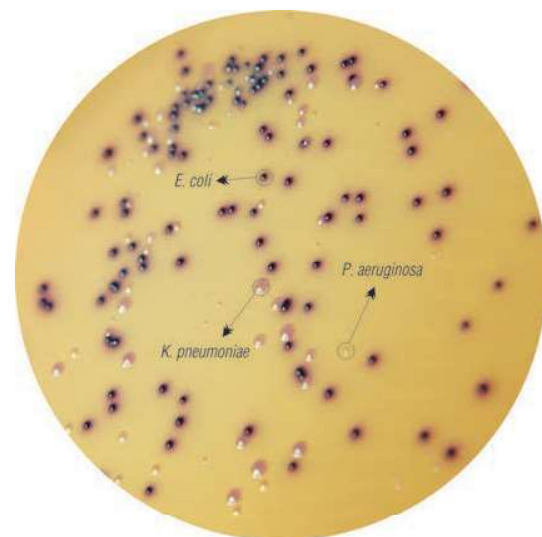
Food and environmental sample

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5, 6).

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



M1293 HiCrome™ ECC Agar

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

HiCrome™ ECC Agar (M1293) is also available as HiCrome™ ECC HiVeg™ Agar (MV1293) wherein all the animal origin nutrients have been replaced by vegetable based nutrients

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Limitations

1. β -glucuronidase is present in 97% of *E. coli* strains, however few *E. coli* may be negative.
2. Certain species of *Shigella* and *Salmonella* are β -glucuronidase positive which may appear as light blue.

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 pink coloured, homogeneous, free flowing powder.
- Gelling** : Firm, comparable with 1.5% Agar gel.
- Colour and Clarity of prepared medium** : Reddish pink coloured, opaque gel forms in Petri plates.
- Reaction** : Reaction of 5.58% w/v aqueous solution at 25°C. pH: 6.8 \pm 0.2
- Cultural Response** : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery of colony	Colour
<i>Escherichia coli</i> (25922) (00013*)	50-100	luxuriant	\geq 70%	blue / purple
<i>Klebsiella pneumoniae</i> (13883) (00097*)	50-100	luxuriant	\geq 70%	rose / pink
<i>Pseudomonas aeruginosa</i> (27853) (00025*)	50-100	good - luxuriant	\geq 70%	straw
<i>Salmonella</i> Enteritidis (13076) (00030*)	50-100	luxuriant	\geq 70%	pink

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 (7, 8).

References

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3. Kilian M. and Bülow P., 1976, Acta. Pathol. Microbiol. Scand., Sect. B, 84:245.
4. Kilian M. and Bülow P., 1979, Acta. Pathol. Microbiol. Scand., Sect. B, 87:271.
5. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., 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.
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Ready Prepared Media			
Code	Product Name	Usage	Packing
Category : HiDip Slides			
HD036	HiDip™ HiCrome™ ECC Agar-HiCrome™ Salmonella Agar	for chromogenic screening of <i>E. coli</i> , coliforms and Salmonella on surfaces or food or water	5 tubes / 10 tubes
HD037	HiDip™ HiCrome™ ECC Agar-PCA w/TTC & Neutralizers	for detection of <i>E. coli</i> , coliforms and for total bacterial count with inactivation of disinfectants	5 tubes / 10 tubes
HD038	HiDip™ HiCrome™ ECC Agar-Baird Parker Agar w/Neutralizers	for detection of <i>E. coli</i> , coliforms and <i>S. aureus</i> with inactivation of disinfectants	5 tubes / 10 tubes