HiCrome™ M-Modified ECO157:H7 Selective Agar Base

Recommended for presumptive enumeration of *Escherichia coli* 0157:H7 by membrane filtration technique.

M1862

Composition **

Ingredients	Grams/Litre
Peptone	5.000
Yeast extract	3.000
Sodium chloride	5.000
Lysine	10.000
Sorbitol	20.000
Dextrose (Glucose)	2.500
Magnesium sulphate	1.500
Sodium deoxycholate	0.150
Sodium glucuronate	0.500
Phenol red	0.120
Chromogenic mixture	0.050
Agar	15.000

Final pH (at 25°C) 7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 62.82 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 45-50°C and aseptically add rehydrated contents of one vial of HiCrome™ ECO157: H7 Selective Supplement, Modified (FD295). Mix well and pour in to sterile Petri plates.

Principle and Interpretation

Escherichia coli O157:H7 belongs to the Enterohemorrhagic *Escherichia coli* (EHEC) group and it predominates as a food borne pathogen. *E.coli* O157:H7 was first recognized as a human pathogen in 1982 when two outbreaks of hemorrhagic colitis were associated with consumption of undercooked ground beef that has been contaminated with this organism (3) that results from the action of a shiga-like toxin (SLT) (1, 7). This medium is recommended for isolation of enteropathogenic *Escherichia coli* O157:H7 in meats, poultry, dairy foods, infant formula, liquid eggs, mayonnaise and apple cider (4, 5). The medium is based on three differential biochemical reactions - lysine decarboxylase (positive for typical EHEC O157 strains), sorbitol fermentation and beta-glucuronidase (2). This medium is also used for the enumeration of β- glucuronidase-positive *E.coli* from foods (6).

Peptone and yeast extract provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential growth nutrients. Sodium chloride maintains the osmotic environment of the medium. Bacteria which were able to grow on this medium will ferment dextrose first. Once dextrose has been depleted, sorbitol positive bacteria will begin to ferment sorbitol, producing a drop in pH of medium, which produces yellow colour to the colony due to phenol red which is a pH indicator. Glucuronidase positive *E.coli* will break down X-Gluc, resulting in the production of an insoluble blue precipitate in the colony. This will combine with the colour of the pH indicator dye to produce a green colony in case of sorbitol positive or lysine negative bacteria. This medium also contains lysine, lysine positive organisms decarboxylates lysine which produces an increase in pH of medium, hence produces pink coloured colonies. Selectivity is achieved through the use of monensin (FD295) which inhibits gram positive bacteria and incubation at 44 - 44.5°C inhibits gram negative bacteria. Most of the other organisms are unable to grow and if any develop yellow colonies.

Type of specimen

Food samples ; Dairy samples

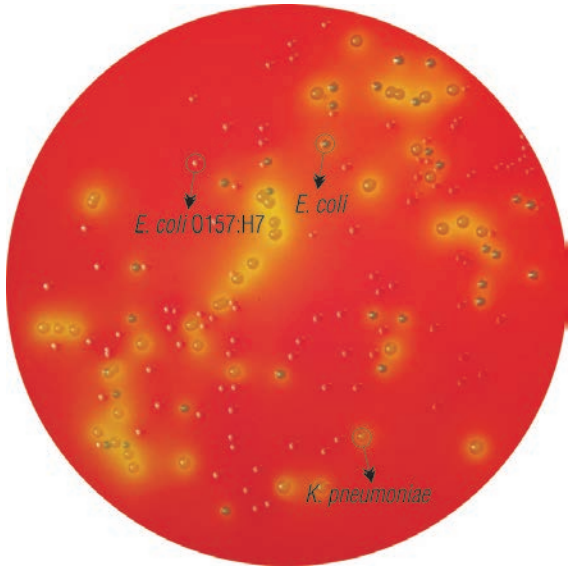
Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3, 8, 9).

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



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Limitations

1. ß-glucuronidase is present in 97% of *E.coli* strains, however few *E.coli* may be negative.
2. Certain species of *Shigella* and *Salmonella* are ß-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 homogeneous free flowing powder

Gelling : Firm, comparable with 1.5 % Agar gel.

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

Reaction : Reaction of 6.28% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2

Cultural Response : Cultural characteristics observed with added HiCrome™ ECO157:H7 Selective Supplement, Modified (FD295), after an incubation at 44 - 44.5°C for 18-24 hours.

Organism (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of Colony (On membrane filter)
Escherichia coli (25922) (00013*)	50-100	luxuriant	>50%	green
Escherichia coli O157:H7 (NCTC 12900)	50-100	luxuriant	≥50%	pink
Klebsiella pneumoniae (13883) (00097*)	50-100	fair	20-30%	yellow
Staphylococcus aureus subsp aureus (25923) (00034*)	$\geq 10^3$	inhibited	0%	-
Enterococcus faecalis (29212) (00087*)	$\geq 10^3$	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 (10, 11).

References

1. Centre for Diseases Control, 1991, Morbid. Mortal, Weekly Rep 40:265.
2. Corry J.E.L, Curtis G.D.W., Baird R.M., Culture Media for Food Microbiology, Progress in Industrial Microbiology, Volume 37.
3. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
4. Entis, P.,and I. Lerner.1997. 24-hour presumptive enumeration of *Escherichia coli* O157:H7 in food using the ISO-GRID method with SD-39 agar.J.Food Prot. 60:883-890.
5. Entis, P.1998. Direct 24-hour presumptive enumeration of *Escherichia coli* O157:H7 in food using the hydrophobic grid membrane filter,followed by serological confirmation : collaborative study. J. AOAC Int. 81:403-418.
6. Entis, P., and I.Lerner. 1998. Enumeration of *β*-glucuronidase positive *E.coli* in foods by using the ISO-GRID method with SD-39 agar.J.Food Prot. 61:913-916.
7. March S. B. and Ratnam S., 1986, J. Clin. Microbiol., 23:869.
8. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
9. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
10. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
11. 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.