HiCrome Modified ECO157:H7 Selective Agar Base

M1862

Hicrome M-Modified EC0157:H7 Selective Agar Base is recommended for presumptive enumeration of EC0157:H7 by membrane filtration technique.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Yeast extract	3.000
Sodium chloride	5.000
Lysine	10.000
Sorbitol	20.000
Dextrose	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 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 (1) that results from the action of a shiga-like toxin (SLT) (2,3).

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 (7). This medium is also used for the enumeration of beta- glucuronidase-positive *E.coli* from foods (6).

Peptic digest of animal tissue and yeast extract provides nitrogenous, carbonaceous compounds 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 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.

Quality Control

Appearance
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

pH
7.00-7.40

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.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony (On membrane filter)
Cultural Response				
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%	green
Escherichia coli O157:H7 NCTC 12900	50-100	luxuriant	>=50%	pink
Klebsiella pneumoniae ATCC 13883	50-100	fair	20-30%	yellow
Staphylococcus aureus ATCC25923	>=10^3	inhibited	0%	
Enterococcus faecalis ATCC 29212	>=10^3	inhibited	0%	

Storage and Shelf Life
Store dehydrated and prepared medium at 2-8°C in tightly closed container. Use before expiry period on the label.

Reference
1.Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., Public Health Association, Washington, D.C.
2.March S. B. and Ratnam S., 1986, J. Clin. Microbiol., 23:869.
3. Centre for Diseases Control, 1991, Morbid. Mortal, Weekly Rep 40:265.
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 #-glucuronidase positive E.coli in foods by using the ISO-GRID method with SD-39 agar.J.Food Prot. 61:913-916.
7.Corry J.E.L, Curtis G.D.W., Baird R.M., Culture Media for Food Microbiology, Progress in Industrial Microbiology, Volume 37.

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