HiCrome™ Enrichment Broth Base for EC 0157:H7

M1598

Recommended for isolation and selective differentiation of *Escherichia coli* O157:H7 from food and environmental samples by chromogenic method.

Composition **

Ingredients	Grams/Litre
Tryptone	10.00
Sorbitol	10.00
Bile salts mixture	1.50
Chromogenic mixture	1.30

Final pH (at 25°C) 7.1 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 11.4 grams in 500 ml distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. For selective isolation of *E.coli* O157:H7, aseptically add the rehydrated contents of 1vial of HiCrome™ ECO157:H7 Selective Supplement I (FD230). Mix well and dispense into sterile test tubes or flasks as desired.

Principle and Interpretation

March and Ratnam (1) reported the inability of *Escherichia coli* O157:H7 to ferment sorbitol while developing Sorbitol MacConkey medium. Subsequently Thomson et al (2) observed the absence of β-glucuronidase activity in *E. coli* O157:H7 from a variety of samples by direct culture.

The medium contains Tryptone that provides nitrogenous, carbonaceous compounds and other essential growth nutrients. Sorbitol is the fermentable carbohydrate, bile salt mixture inhibits most of the gram-positive organisms. Addition of tellurite (FD230) makes the medium more specific and selective. The bluish colour development of *E. coli* and *Klebsiella* in the medium is due to the enzymes β-D-galactosidase and β-D-glucuronidase respectively that cleaves the chromogenic substrates present in chromogenic mixture. However *E. coli* O157:H7 gives a purple colour to the medium due to the absence of β-glucuronidase and its inability to ferment sorbitol.

Type of specimen

Food and Environmental samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (3, 4, 5). 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. Certain species of *Shigella* and *Salmonella* are ß-glucuronidase positive which may appear as light blue.
2. Further biochemical test must be carried out for confirmation.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the recommended temperature.

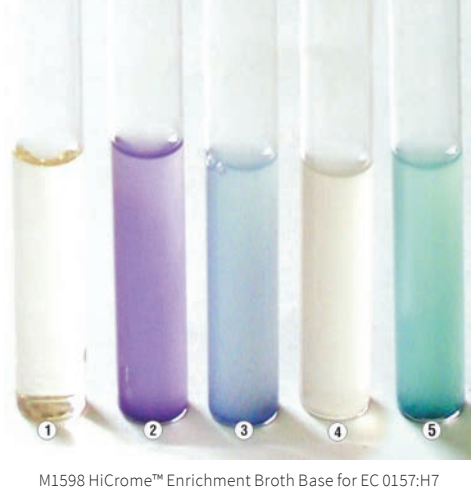
Quality Control

Appearance of Powder : Cream to yellow coloured, homogeneous, free flowing powder.

Colour and Clarity of prepared medium : Light yellow coloured, clear solution without any precipitate.

Reaction : Reaction of 2.28% w/v aqueous solution at 25°C. pH : 7.1 ± 0.2.

Cultural Response : Cultural characteristics observed with added HiCrome™ EC O157:H7 Selective Supplement I (FD230) after an incubation at 35-37°C for 18-24 hours.



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1. Control
2. *E. coli* 0157:H7 (NCTC 12900)
3. *Escherichia coli* (ATCC 25922 (00013*)
4. *Cronobacter sakazakii* (ATCC 12868)
5. *Klebsiella pneumoniae* (ATCC 13883)

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Organisms (ATCC)	Inoculum (CFU)	Growth	Colour of Medium	Growth«	Colour of Medium«
Escherichia coli O157: H7 (NCTC 12900)	50-100	good-luxuriant	purple#	good-luxuriant	purple#
Escherichia coli (25922) (00013*)	50-100	good-luxuriant	blue#	inhibited	-
****Cronobacter sakazakii*** (12868)	50-100	good-luxuriant	white#	none-poor	colourless#
Klebsiella pneumoniae (13883) (00097*)	50-100	good-luxuriant	bluish green	good	bluish green#
Salmonella Enteritidis (13076) (00030*)	50-100	good-luxuriant	colourless#	good	colourless#
Shigella flexneri (12022) (00126*)	50-100	good-luxuriant	colourless	inhibited	-
Enterococcus faecalis (29212) (00087*)	50-100	good	-	inhibited	-
Staphylococcus aureus subsp aureus (25923) (00034*)	$\geq 10^3$	inhibited	-	inhibited	-

KEY : « : after addition of HiCrome™ ECO157:H7 Selective Supplement I (FD230)
: may show slight precipitation of growth
** : Formerly known as *Enterobacter sakazakii*
* = 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 (6, 7).

References

1. March S. B. and Ratnam S., (1986), J. Clin. Microbiol. 23, 869 - 872.
2. Thompson et al. (1990), J. Clin. Microbiol. 29, 2165 - 2168.
3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
4. 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.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
7. 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.