CM 22,190 –CHROMOGENIC ECO157:H7 SELECTIVE AGAR BASE, MODIFIED

INTENDED USE

For presumptive enumeration of Escherichia coli 0157:H7 by membrane filtration technique from food samples.

PRODUCTSUMMARY AND EXPLANATION

Escherichia coliO157:H7belongs 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 that results from the action of a shiga-like toxin (SLT). 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. The medium is based on three differential biochemical reactions - lysine decarboxylase (positive for typical EHEC O157 strains), sorbitol fermentation and beta-glucuronidase. This medium is also used for the enumeration of beta-glucuronidase-positive E.coli from foods.

COMPOSITION

Ingredients	Gms / Ltr
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 glucuronate	0.500
Sodium deoxycholate	0.150
Phenol red	0.120
Chromogenic mixture	0.050
Agar	15.000

PRINCIPLE

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 organism's 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.

INSTRUCTION FOR USE

Dissolve 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 Chromogenic ECO157: H7 Selective Supplement, Modified.
Mix well and pour in sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Light yellow to pink homogeneous free flowing powder

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

pH (at 25°C) : 7.2 ± 0.2

INTERPRETATION

Culturalcharacteristics observed with added Chromogenic ECO157:H7 Selective Supplement, Modified, after an incubation.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth	Color of the medium	Recovery	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	luxuriant	Green	>=50%	44 - 44.5°C	18-24 Hours
Staphylococcus aureus	25923	50-100	Inhibited	-	-	44 - 44.5°C	18-24 Hours
Klebsiella pneumoniae	13883	50-100	Fair	Yellow	20-30%	44 - 44.5°C	18-24 Hours
Enterococcus faecalis	29212	>=10^3	Inhibited	-	-	44 - 44.5°C	18-24 Hours

PACKAGING:

Inpacksizeof100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

Afteruse,prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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.

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

*For Lab Use Only