# HiCrome™ Modified ECO157:H7 Selective Agar Base

M1862

## Intended Use:

Recommended for presumptive enumeration of *Escherichia coli* 0157:H7 by membrane filtration technique from food samples. It can also be used for isolation from clinical samples.

## Composition**

| Ingredients | g / L |
|---|---|
| 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 purified / 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 MSN Selective Supplement, (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 ß-glucuronidase (6). This medium is also used for the enumeration of ß-glucuronidase-positive *E.coli* from foods (7).

Peptone and yeast extract provides nitrogenous, carbonaceous compounds, long chain amino acids, vitamins 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 grows, develop yellow colonies.

## Type of specimen

Clinical samples - Stool, urine, etc.; Food and dairy samples

## Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,10,11).

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

In Vitro diagnostic use only. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1. Slight variation in colour may be observed due to strain variation.
2. Some species may show poor growth due to nutritional variations.

## PerformanceandEvaluation

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

## QualityControl

**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 MSN Selective Supplement (FD295), after an incubation at 44 - 44.5°C for 18-24 hours.

| Organism | Inoculum (CFU) | Growth | Recovery | Colour of Colony (On membrane filter) |
|---|---|---|---|---|
| *Escherichia coli O157:H7 NCTC 12900* (00014*) | 50-100 | luxuriant | >=50% | pink |
| *Staphylococcus aureus subsp. aureus* ATCC 25923 (00034*) | >=$10^4$ | inhibited | 0% | |
| *Enterococcus faecalis* ATCC 29212 (00087*) | >=$10^4$ | inhibited | 0% | |
| *Klebsiella pneumoniae ATCC 13883* (00097*) | 50-100 | fair | 20-30% | yellow |
| *Escherichia coli ATCC 25922* (00013*) | 50-100 | luxuriant | >=50% | green |

Key : (*) Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 15-25°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. 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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

## Reference

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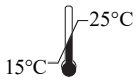
IVD *In vitro* diagnostic medical device




Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

Do not use if package is damaged

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