HiColiform™ Broth, Modified

Recommended for the detection and confirmation of *Escherichia coli* and total coliforms from water samples, using a combination of chromogenic and fluorogenic substrates.

M1850

Composition **

Ingredients	Grams/Litre
Peptone	5.000
Sodium chloride	5.000
Potassium sulfate	1.000
Dipotassium hydrogen phosphate	4.000
Potassium dihydrogen phosphate	1.000
Sodium lauryl sulphate (SLS)	0.100
Sodium puruvate	1.000
Chromogenic substrate	0.100
Fluorogenic substrate	0.100
IPTG	0.100

Final pH (at 25°C) 6.8±0.2
** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17.4 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense in tubes or flasks or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle and Interpretation

HiColiform™ Broth, Modified was designed for detection and confirmation of *Escherichia coli* and total coliforms from water samples using a combination of chromogenic and fluorogenic substrates. *Escherichia coli* can be distinguished from other coliforms by its unique ability to fluoresce in the presence of fluorogenic substrate (1, 2). The fluorogenic substrate is split by enzyme β-glucuronidase especially present in *Escherichia coli*. The reaction is indicated by the development of a blue fluorescence under UV light. The presence of total coliforms is indicated by blue-green colourations due to the cleavage of the chromogenic substrate. IPTG amplifies enzyme synthesis and increases the activity of β -galactosidase.

Peptone provides essential growth nutrients and is useful for the simultaneous detection of indole production. The phosphate salts provide buffering action for rapid growth of coliforms. Sodium chloride helps to maintain the osmotic balance. Sodium lauryl sulphate makes the medium selective by inhibiting accompanying microflora, especially the gram-positive organisms.

Type of specimen

Water samples

Specimen Collection and Handling

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

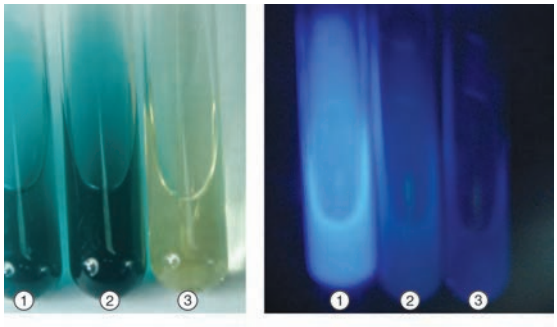
After use, contaminated materials must be sterilized by autoclaving before discarding.

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. β-glucuronidase especially present in 97% of *Escherichia coli,* however few *E. coli* may be negative.
2. Some species may show poor growth due to nutritional variations.



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erichia coli 2. *Enterobacter aerogenes* 3. Control

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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 homogeneous free flowing powder

Colour and Clarity of prepared medium : Light yellow coloured, clear to slightly opalescent solution in tubes

Reaction : Reaction of 1.74% w/v aqueous solution at 25°C. pH : 6.8±0.2

Cultural Response : Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism (ATCC)	Inoculum	Growth	Colour of medium	Fluorescence (under uv)
#Klebsiella aerogenes (13048) (00075*)	50-100	luxuriant	blue-green	negative reaction
Escherichia coli (25922) (00013*)	50-100	luxuriant	blue-green	positive reaction

Key : * : Corresponds to WDCM number
: Formerly known as *Enterobacter aerogenes*

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 (3, 4).

References

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