

EC Blue HiVeg™ Broth

MV1768

Intended Use:

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

Composition**

Ingredients	g / L
HiVeg™ peptone	5.000
Sodium chloride	5.000
Sodium pyruvate	1.000
Potassium dihydrogen phosphate	1.000
Dipotassium hydrogen phosphate	4.000
Potassium nitrate	1.000
Sodium lauryl sulphate (SLS)	0.100
Isopropyl-β-D-1-thiogalactopyranoside (IPTG)	0.100
X-Gal	0.100
4-Methylumbelliferyl β-D-Glucuronide (MUG)	0.100
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17.40 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Dispense into tubes or flasks or as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

EC Blue Broth was designed for detection and confirmation of *Escherichia coli* and other coliforms. *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, MUG 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 (X-Gal). IPTG amplifies enzyme synthesis and increases the activity of β-galactosidase. EC Blue HiVeg™ Broth is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. HiVeg™ 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

Food samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (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. This medium is general purpose medium and may not support the growth of fastidious organisms.
2. Further biochemical and serological tests must be carried out for further identification.
3. Some organism may show poor growth due to nutritional variation.

Performance and Evaluation

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

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Cream coloured clear solution having slight precipitate in tubes

Reaction

Reaction of 1.74 % w/v aqueous solution at 25°C. pH : 7.1±0.2

pH

6.90-7.30

Cultural Response

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

Organism	Inoculum (CFU)	Growth	Colour change in medium	Fluorescence under UV light
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	Bluish green	positive reaction
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	luxuriant	blue	negative reaction
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	blue	negative reaction
<i>Citrobacter freundii</i> ATCC 8090	50-100	luxuriant	Bluish green	negative reaction
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	colourless	negative reaction

Key : (*) Corresponding WDCM numbers. (#) Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store dehydrated 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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- Feng P.C.S. and Hartman P.A. ,1982, J.Appl. Environmental Microbiol. 43. 1320-1323.
- Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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- Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- 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.

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