



Buffered Peptone Water w / pyruvate

M1851

Buffered Peptone Water w/ Pyruvate is recommended for the isolation of Enterohemorrhagic *E. coli* (EHEC).

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Lactose	10.000
Yeast extract	6.000
Casein acid hydrolysate	5.000
Sodium chloride	5.000
Sodium phosphate, dibasic	3.600
Potassium phosphate, monobasic	1.500
Sodium pyruvate	1.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21.05 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 and aseptically add the rehydrated contents of one vial of Acriflavin-Cefsulodin-Vancomycin Supplement (ACV Supplement, FD284).

Principle And Interpretation

Enterohaemorrhagic *E. coli* (EHEC) can cause severe foodborne disease. EHEC is the primary cause of hemorrhagic colitis. This infection can also lead to hemolytic uremic syndrome (1). It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Its significance as a public health problem was recognized in 1982, following an outbreak in the United States of America. EHEC produces toxins, known as verotoxins or Shiga-like toxins because of their similarity to the toxins produced by *Shigella dysenteriae* (2).

The media contains Casein enzymic hydrolysate, Casein acid hydrolysate and Yeast extract as carbon and nitrogen sources. Phosphates buffer the medium. The phosphate buffer system prevents bacterial damage due to changes in the pH of the medium. Sodium chloride maintains the osmotic balance. Lactose serves as a carbon source.

Antibiotic supplement contains Acriflavin, Cefsulodin, and Vancomycin which effectively suppress the normal flora while allow the growth of *Escherichia coli O157:H7* (1).

After the sample has been prepared, material will be placed in Buffered Peptone Water w/Pyruvate and incubated at 37 ± 1 °C for 5 hours. Next add rehydrated contents of one vial of Acriflavin-Cefsulodin-Vancomycin Supplement (ACV Supplement, FD284) and incubate at 42 ± 1 °C for 18-24 hours.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Amber coloured, clear solution without any precipitate

Reaction

Reaction of 4.21% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed with an added Acriflavin-Cefsulodin-Vancomycin Supplement (ACV Supplement, FD284) after an incubation at 42 ± 1 °C for 18-24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response <i>Escherichia coli</i> O157:H7 (NCTC 12900)	50-100	good	40-50%
<i>Staphylococcus aureus</i> (25923)	$\geq 10^3$	inhibited	0%
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	good	40-50%
<i>Enterococcus faecalis</i> (29212)	$\geq 10^3$	inhibited	0%
<i>Escherichia coli</i> (25922)	$\geq 10^3$	inhibited	0%

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm
2. www.who.int/mediacentre/factsheets/fs125/en/

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