

# **Buffered Peptone Water w/ Casein**

# **Intended Use:**

Recommended as pre-enrichment medium for increasing the recovery of injured *Salmonella* species from food particularly cocoa, chocolate, confectionary etc. prior to selective enrichment and isolation.

# Composition\*\*

Ingredients	Gms / Litre
Tryptone	10.000
Sodium chloride	5.000
Disodium hydrogen phosphate dodecahydrate	9.000
Potassium dihydrogen phosphate	1.500
M-Protein Powder #	50.000
Final pH ( at 25°C)	7.0±0.2
**Formula adjusted, standardized to suit performance parameters	

# - Equivalent to Casein

### Directions

Suspend 70.07 grams (equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Mix well and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

# **Principle And Interpretation**

Microorganisms that are subjected to environmental stress may become structurally or metabolically damaged or injured. These microorganisms are unable to replicate in selective environments. Therefore these injured organisms

must be resuscitated or permitted to repair the damage by incubation in an appropriate, non-selective environment (9). Edel and Kampelmacher (3) noted that sub-lethal injury to *Salmonella* may occur in many food preservation processes. Enriching injured cells in Lactose Broth (pH 6.9) may be further detrimental to their recovery (2). Pre-enrichment in Buffered Peptone Water (M1494I) at 35°C for 18-24 hours results in repair of injured cells (7). The buffering system prevents bacterial damage due to change in the pH of the medium. Recently ISO committee has also recommended this pre-

enrichment medium for the detection of *Enterobacteriaceae* from food stuffs and other materials (4). Addition of 50g/l M-Protein Powder or skimmed milk is recommended for testing cocoa and cocoa containing products (more than 20%) as recommended by ISO (4). The addition of M-Protein Powder is necessary to inhibit bactericidal substances present in cocoa or cocoa containing products. The sample is enriched in Buffered peptone water w/casein (M1919), further enriched in selective enrichment media and then subcultured on XLD Agar (M031I).

### **Type of specimen**

Food and dairy samples - cocoa, chocolate, confectionary, milk and milk products, etc. (4)

### **Specimen Collection and Handling**

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,10). 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. Further enrichment in selective medium is required and then subcultured on XLD Agar (M031I).

# M1919

#### **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 opaque solution

#### Reaction

Reaction of 7.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

#### pН

6.80-7.20

#### **Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.(Recovery is observed on XLD Agar, M0311)

Organism	Growth	Inoculum (CFU)	Recovery
Salmonella Enteritidis ATC 13076 (00030*)	Cluxuriant	50-100	>=50%
Salmonella Typhi ATCC 6539	luxuriant	50-100	>=50%
Salmonella Typhimurium ATCC 14028 (00031*)	luxuriant	50-100	>=50%
Salmonella Choleraesuis ATCC 12011	luxuriant	50-100	>=50%
Salmonella Paratyphi A ATCC 9150	luxuriant	50-100	>=50%
Salmonella Paratyphi B ATCC 8759	luxuriant	50-100	>=50%

Key : (\*) Corresponding WDCM numbers.

#### **Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 (5,6).

#### Reference

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- 4. International Organization for Standardization (ISO), 2002, Draft ISO/DIS, 6579.
- 5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2<sup>nd</sup> 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.

7. Sadovski A. Y., 1977, J. Food Technol., 12.85.

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9. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.

10. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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