# BUFFERED PEPTONE WATER

**Dehydrated and ready-to-use culture medium**

## 1 - INTENDED USE

Buffered Peptone Water is used as non-selective pre-enrichment medium and diluent in procedures for the detection and enumeration of bacteria, and pathogens such as *Salmonella, Cronobacter, Listeria monocytogenes, Listeria* spp. *Enterobacteriaceae*, in foods, animal feeding stuffs, water and other materials.
The medium complies with the specifications given by ISO 6579, ISO 11290-2, ISO 22964, ISO 21528-1, ISO 6887, ISO 19250.

## 2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

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|---|---|
| Peptone° | 10.0 g |
| Sodium chloride | 5.0 g |
| Disodium hydrogen phosphate anhydrous | 3.5 g ^ |
| Monopotassium phosphate | 1.5 g |

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
°Includes enzymatic digest of casein
^ equivalent to disodium hydrogen phosphate dodecahydrate 9 g/L

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Buffered Peptone Water is the historical formulation included in the Biolife catalogue since the late 1960s, prepared with a universal peptone which includes enzymatic digest of casein, particularly rich in nutrients and with a buffer system that allows optimal recovery of microorganisms even when present in the sample in a very low number or sub-lethally injured.
Buffered Peptone Water can be used as:

- a non-selective pre-enrichment medium for the detection of *Salmonella* according to ISO 6579,[1,2] in samples of the food chain and according to ISO 19250 in water samples;[3]
- a non-selective pre-enrichment medium for the detection of *Cronobacter* according to ISO 22964 in samples of the food chain;[4]
- a non-selective enrichment medium for the detection of *Enterobacteriaceae* according to ISO 21528 in samples of the food chain;[5]
- a diluent for the enumeration of *Listeria monocytogenes* and of *Listeria* spp. according to ISO 11290-2;[6]
- a diluent for the enumeration of microorganisms according to ISO 6887.[7]

Peptone provides carbon, nitrogen, vitamins and minerals for microbial growth, sodium chloride maintains the osmotic balance, while phosphates buffer the medium at pH 7.0. The pH 7.2 medium variant according to FDA-BAM is available under code 401278S.

## 4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 20 g in 1000 mL of cold purified water. Mix thoroughly and warm gently to completely dissolve the powder, if necessary. Distribute into flasks or tubes of suitable capacity and sterilise in the autoclave at 121°C for 15 minutes.

## 5 - PHYSICAL CHARACTERISTICS

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|---|---|
| Dehydrated medium appearance | beige, fine, homogeneous, free-flowing powder |
| Prepared medium appearance | pale yellow, limpid |
| Final pH at 20-25 °C | 7.0 ± 0.2 |

## 6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| Buffered Peptone Water | Dehydrated medium | 4012782<br>4012784 | 500 g (25 L)<br>5 kg (250 L) |
| Buffered Peptone Water | Ready-to-use medium in tubes | 551278 | 20 x 9 mL |
| Buffered Peptone Water | Ready-to-use medium in flasks | 5112782<br>5112783 | 6 x 90 mL<br>6 x 225 mL |

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, bottles, ancillary culture media and reagents.

## 8 – SPECIMENS

Waters, foods, animal feeding stuffs, environmental samples in the area of food production and food handling. Refer to applicable International Standards for the collection, transport, storage of samples and operate in accordance with good laboratory practice. [1-7]

## 9 - TEST PROCEDURE

For details of sample preparation and enrichment, refer to the Standards cited according to the intended use.[1-7]
Pre-enrichment for *Salmonella* detection: in general, 225 mL of Buffered Peptone Water are inoculated with 25 g of the test portion, then incubated between 34°C and 38°C for 18 h ± 2 h. It is permissible to store the pre-enriched sample after incubation at 2-8°C for a maximum of 72 h.

## 10 - READING AND INTERPRETATION

Microbial growth in Buffered Peptone Water is evidenced by the development of turbidity in the broth.

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## 11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. The choice of strains depends on the intended use. Consult the suitable ISO Standard.[8]

## 12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated and ready to use Buffered Peptone Water (Test Batch: TB), is tested for productivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes and incubating at 35-37° for 18-24 hours and recording the highest dilution showing growth in Reference Batch ($Gr_{RB}$) and in Test Batch ($Gr_{TB}$).
Productivity is tested with the following strains: *E.coli* ATCC 8739, *S.*Typhimurium ATCC 14028, *S.*Enteritidis ATCC 13076, *C.sakazaki* ATCC 29544, *C.muytjensis* ATCC 51329. The productivity index $Gr_{RB}$-$Gr_{TB}$ for each test strain shall be ≤ 1.
Buffered Peptone Water is also evaluated for test strains survival after 1 hour at 20°C into the device with subculture and enumeration in Tryptic Soy Agar. The ratio A/C (CFU obtained after 1 hour of incubation of the inoculated medium/CFU obtained immediately after the inoculation of the medium) shall be between 0.7 and 1.3 for the following strains: *E.coli* ATCC 8739, *L.monocytogenes* ATCC 13932, *S.aureus* ATCC 25923.

## 13 – LIMITATIONS OF THE METHOD

- Buffered Peptone Water is a general-purpose medium without selective properties. Suitable selective liquid and solid media must be inoculated with the growth obtained in Buffered Peptone Water.
- The test sample may increase the turbidity of the medium although bacterial growth is not present. Subculture to appropriate media is necessary to verify growth of organisms.

## 14 - PRECAUTIONS AND WARNINGS

- These products are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The ante and post mortem controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that the product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap flasks and tubes to prevent injury due to breakage of glass.
- Ready-to-use flasks and tubes of Buffered Peptone Water are subject to terminal sterilization by autoclaving.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized tubes/flasks inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

## 15 - STORAGE CONDITIONS AND SHELF LIFE

**Dehydrated medium**

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (tubes/flasks) and the applied storage conditions (temperature and packaging). According to ISO 6579-1 autoclaved Buffered Peptone Water may be stored in closed containers at 2-8 °C for up to six months.

**Ready-to-use medium in tubes and bottles**

Upon receipt, store tubes and flasks in their original pack at 2-8°C away from direct light. If properly stored, the tubes and the flasks may be used up to the expiration date. Do not use the tubes and the flasks beyond this date. Tubes and flasks from opened secondary packages can be used up to the expiration date. Opened tubes and flasks must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes or flasks with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

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Content sufficient for <n> tests



## 16 - REFERENCES

1. ISO 6579-1:2017/AMD 1:2020 Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. - Amendment 1: Broader range of incubation temperatures, amendment to the status of Annex D, and correction of the composition of MSRV and SC
2. ISO/TS 6579-2:2012 Microbiology of food and animal feed - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 2: Enumeration by a miniaturized most probable number technique.
3. ISO 19250:2010 Water quality - Detection of Salmonella spp.
4. ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of Cronobacter spp.
5. ISO 21528-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae -Part 1: Detection of Enterobacteriaceae
6. ISO 11290-2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. - Part 2: Enumeration method
7. ISO 6887-1:2017 Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions
8. ISO 11133:2014 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media

**TABLE OF APPLICABLE SYMBOLS**

| | | | | | |
|---|---|---|---|---|---|
| REF or REF Catalogue number | LOT Batch code | Manufacturer | This side up | Store in a dry place | Fragile |
| Temperature limitation | Σ | Consult Instructions for Use | Use by | Keep away from direct light | For single use only |

**REVISION HISTORY**

| Version | Description of changes | Date |
|---|---|---|
| Revision 2 | Updated layout and content | 2022/06 |

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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