



BUFFERED PEPTONE WATER (CASEIN)

Dehydrated culture medium

1 - INTENDED USE

Buffered Peptone Water (Casein) 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* in food and 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)

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate anhydrous	3.5 g [^]
Potassium dihydrogen phosphate	1.5 g

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

[^] equivalent to disodium hydrogen phosphate dodecahydrate 9 g/L

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Most of the International Standards, recommending the use of Buffered Peptone Water, indicate as the ingredient of animal origin to be included in the formula, a material with the generic term "Peptone" and as an example cite enzymatic digest of casein.¹⁻⁶

The ISO 19250 Standard for the detection of *Salmonella* in water, recommending the use of Buffered Peptone Water, explicitly mentions enzymatic digest of casein as the ingredient of animal origin to be included in the formula.³

Buffered Peptone Water (Casein) is manufactured with a selected 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 (Casein) 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;⁷
- a non-selective enrichment medium for the detection of *Enterobacteriaceae* according to ISO 21528;⁴
- a diluent for the enumeration of *Listeria monocytogenes* and of *Listeria* spp. according to ISO 11290-2;⁵
- a diluent for the enumeration of microorganisms according to ISO 6887.⁶

The enzymatic digest of casein provides carbon, nitrogen, vitamins and minerals for microbial growth, sodium chloride maintains the osmotic balance, while phosphates buffer the medium at pH 7.0.

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

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 (Casein)	Dehydrated medium	401278C2	500 g (25 L)
		401278C4	5 kg (250 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile loops, swabs 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 standards¹⁻⁷ for the collection, transport, storage of samples and operate in accordance with good laboratory practice.

9 - TEST PROCEDURE

For details of sample preparation and enrichment, please refer to the standards cited according to the intended use.¹⁻⁸

For the pre-enrichment of *Salmonella* in water Buffered Peptone Water (Casein) is inoculated at ambient temperature with a known volume of the sample or its dilutions, then incubated at 36 ± 2 °C for 18 h ± 2 h. Larger volumes can be concentrated using membrane filtration and the membrane filter is then added to Buffered Peptone Water.

10 - READING AND INTERPRETATION

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

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 Standard cited.⁹

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Buffered Peptone Water Casein (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 (G_{RB}) and in Test Batch (G_{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 G_{RB}/G_{TB} for each test strain shall be ≤ 1 .

Buffered Peptone Water Casein 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 (Casein) 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 (Casein).
- 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

- This product is for microbiological control and for professional use only; it is 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.
- 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 plates 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 Sheet of the product 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

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.










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 21528-1:2017 Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae -Part 1: Detection of Enterobacteriaceae
5. 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
6. 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
7. ISO 22964:2017 Microbiology of the food chain - Horizontal method for the detection of Cronobacter spp.
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	 Store in a dry place	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	

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.

