PHARMACOPOEIA DILUENT

BUFFERED SODIUM CHLORIDE-PEPTONE SOLUTION

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Liquid medium for the test strains and samples suspension and dilution in the microbiological examination of non-sterile pharmaceutical products according to European Pharmacopoeia.

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

Potassium dihydrogen phosphate	3.56 g
Disodium hydrogen phosphate anhydrous	5.76 g^
Sodium chloride	4.30 g
Tryptone	1.00 g

^ Equivalent to 7.2 g of disodium hydrogen phosphate dihydrate
*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Pharmacopoeia Diluent, also known as "buffered sodium chloride-peptone solution", is an isotonic diluent prepared according to the formulation reported by European Pharmacopoeia.[1]
It is recommended: a) to make the microbial suspension for growth promotion test, b) to dissolve and dilute the product to be tested, c) supplemented with specific compounds, for the removal of the antimicrobial activity of disinfectants.
Pharmacopoeia Diluent maintains the viability of microorganisms during sample preparation without supporting growth. It contains a low concentration of peptone and sodium chloride that provides osmotic stability. Phosphates are used as buffering agents to control the pH in the medium.

4 – DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 14.6 g in 1000 mL of cold purified water. Heat to dissolve, distribute and sterilise by autoclaving at 121°C for 15 minutes.
If required add the suitable neutralising compounds. A typical neutralising diluent has the following formulation:

Pharmacopoeia Diluent	1000 mL
Polysorbate 80	30 g
Lecithin (egg)	3 g
Histidine HCl	1 g

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	pale yellow, limpid
Final pH at 20-25 °C	7.0 ± 0.1

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Pharmacopoeia Diluent	Dehydrated medium	4013952	500 g (34.2 L)
Pharmacopoeia Diluent	Ready-to-use flasks	5113952	6 x 90 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, microbiological tubes and flasks, ancillary culture media and reagents.

8 - SPECIMENS

Non-sterile pharmaceutical products and medical devices. Refer to applicable International Standards and regulations and operate in accordance with good laboratory practice for sample collection, storage and transport to the laboratory.

9 - TEST PROCEDURE, READING AND INTERPRETATION

Use Pharmacopoeia Diluent to make test strains suspensions. Use the suspensions within 2 hours or within 24 hours if stored at 2-8°C.
Dissolve o dilute (usually a 1:10 dilution is prepared) the sample in the medium. If necessary, prepare further dilutions in the same diluent.
For details on test methods for the examination of non-sterile pharmaceutical products consult the current edition of European Pharmacopoeia and the instructions for use of relevant isolation and enumeration media.

10 - 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, it is responsibility of the end-user to perform Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS
S.aureus ATCC 6538	120 min at room temperature	± 30% original count (subculture in Tryptic Soy Agar)
E. coli ATCC 8739	120 min at room temperature	± 30% original count (subculture in Tryptic Soy Agar)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

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11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated and ready to use Pharmacopoeia Diluent, is assessed for test strains survival by comparing the results with a previously approved Reference Batch.
Pharmacopoeia Diluent is evaluated for test strains survival after 2 hours at room temperature into the tubed broth with subculture and enumeration in Tryptic Soy Agar. The ratio A/C (CFU obtained after 2 hours 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, *S.aureus* ATCC 6538, *P.aeruginosa* ATCC 9027, *C.albicans* ATCC 10231.

12 - PRECAUTIONS AND WARNINGS

- This culture medium 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 this 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 to prevent injury due to breakage of glass.
- Ready-to-use flasks 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 medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium 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 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.

13 - STORAGE CONDITIONS AND SHELF LIFE

Ready-to-use medium in flasks

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

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/bottles) and the applied storage conditions (temperature and packaging).

14 - REFERENCES

1. European Pharmacopoeia 11th Edition, 2022, Vol. 1; 2.6.13 Microbiological Examination of non-sterile products: test for specified micro-organisms: 01/2021:20631

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/11

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

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