

MAXIMUM RECOVERY DILUENT (PEPTONE SALT SOLUTION)

Dehydrated and ready-to-use culture medium

1 - INTENDED USE

Isotonic diluent for the preparation of the initial suspension and dilutions of foods and animal feed stuffs for microbiological examination.

2 - COMPOSITION - TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

DEHYDRATED AND READY-TO-USE TUBES AND FLASKS

| | |
|----------------------------|-------|
| Enzymatic digest of casein | 1.0 g |
| Sodium chloride | 8.5 g |

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Maximum Recovery Diluent, also known as peptone salt solution, is formulated as recommended by ISO 6887¹. It is an isotonic diluent for maximal recovery of microorganisms from samples of the food chain.

The presence of low levels of enzymatic digest of casein in the diluent at a pH 7.0 protects the bacteria and does not allow them to multiply for at least 1-2 hours during the dilution phase. Sodium chloride at physiological strength maintains the osmotic equilibrium contributing to the recovery of microorganisms.

4 - DIRECTIONS FOR DEHYDRATED MEDIUM PREPARATION

Suspend 9.5 g in 1000 mL of cold purified water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Distribute 9 mL in tubes or 90 mL or 225 mL in flasks and sterilise in the autoclave at 121°C for 15 minutes. Cool to room temperature before the use.

5 - PHYSICAL CHARACTERISTICS

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

6 - MATERIALS PROVIDED - PACKAGING

| Product | Type | REF | Pack |
|--------------------------|-------------------------------|---------|----------------|
| Maximum Recovery Diluent | Dehydrated medium | 4016912 | 500 g (52.6 L) |
| | | 4016914 | 5 kg (526 L) |
| Maximum Recovery Diluent | Ready-to-use medium in tubes | 551691 | 20 x 9 mL |
| Maximum Recovery Diluent | Ready-to-use medium in flasks | 5116912 | 6 x 90 mL |
| | | 5116913 | 6 x 225 mL |

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, sterile 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. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.¹

9 - TEST PROCEDURE

The working procedure described here is a summary taken from ISO 6887¹. For operational details please refer to the cited Standard.

1. Weigh a mass m g or measure a volume V mL (minimum 10 g or 10 mL, unless otherwise stated by specific method), to a tolerance of $\pm 5\%$, representative of the test sample.
2. Add a quantity of Maximum Recovery Diluent equal to $9 \times m$ g or $9 \times V$ mL, to a tolerance of $\pm 2\%$, to prepare the initial suspension (primary decimal dilution).
3. Homogenize the mixture.
4. Allow large particles to settle, if necessary, for up to 15 min. Filtration systems giving equivalent results, such as plastic bags with integral filter liners, may also be used.
5. For a decimal dilution series for use in enumeration tests, transfer, using a pipette, $1 \text{ mL} \pm 0.02 \text{ mL}$ of the initial suspension into a tube containing 9 mL of Maximum Recovery Diluent and mix thoroughly (10^{-2} dilution).
6. If necessary, repeat these operations using the 10^{-2} and further dilutions to obtain 10^{-3} , 10^{-4} etc. dilutions, until the appropriate number of microorganisms has been obtained.
7. The time between the end of the preparation of the initial suspension and the moment when the inoculum comes into contact with the final culture medium shall not exceed 45 min. Additionally, the time between the preparation of the initial suspension and the beginning of preparation of any subsequent dilutions shall not exceed 30 min.
8. Use initial suspension and decimal dilutions for the purposes of specific microbiological analyses

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, 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. Here below are listed some test strains useful for the quality control of medium.



CONTROL STRAINS
S. aureus ATCC 25923
E. coli ATCC 25922

INCUBATION T° / T / ATM
 60 min at room temperature
 60 min at room temperature

EXPECTED RESULTS
 ± 30% original count (subculture in Tryptic Soy Agar)
 ± 30% original count (subculture in Tryptic Soy Agar)

ATCC is a trademark of American Type Culture Collection

11 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated and ready to use Maximum Recovery Diluent (Test Batch: TB), is assessed for test strains survival by comparing the results with a previously approved Reference Batch (RB).

Maximum Recovery Diluent is 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 25922, *S. aureus* ATCC 25923.

12 – LIMITATIONS OF THE METHOD

- 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.

13 - PRECAUTIONS AND WARNINGS

- This culture medium is for laboratory use 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 or tubes to prevent injury due to breakage of glass.
- Ready-to-use flasks and tubes are subject to terminal sterilization by autoclaving.
- Each ready-to-use tube and flask of this culture medium is for single use only.
- 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 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.

14 - 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).

Ready-to-use medium in tubes and flasks

Upon receipt, store tubes and flasks in their original pack at +2°C /+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).

15 - REFERENCES

- 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.

TABLE OF APPLICABLE SYMBOLS

| | | | | | |
|---|--|--|--|---|---|
|  REF or REF Catalogue number |  LOT Batch code |  Manufacturer |  This side up |  Store in a dry place |  Fragile |
|  Temperature imitation |  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 2 | Updated layout and content | 2022/08 |

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

