TRYPTIC SOY BROTH γ- IRRADIATED

Dehydrated culture medium

1 - INTENDED USE

Gamma-irradiated general-purpose medium, for the cultivation of microorganisms. For the microbiological validation of aseptic filling processes.

2 - COMPOSITION -TYPICAL FORMULA *

(AFTER RECONSTITUTION WITH 1 L OF WATER)

Pancreatic digest of casein	17.0 g
Soy peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose	2.5 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

Tryptic Soy Broth is a general-purpose medium that supports the growth of a wide variety of aerobic and facultative anaerobic bacteria and fungi.[1] The medium is prepared according to the formulation recommended by the current European Pharmacopoeia and complies with the quality specifications reported therein.[2]
Tryptic Soy Broth γ-irradiated in 500 g and 5 kg packs is irradiated with gamma rays at a minimum dose of 25 kGy and maximum of 35 kGy, is cold filterable and triple-bagged allowing for safe introduction into controlled areas. Different doses can be applied based on specific agreements with the customer.
The broth is suitable for monitoring microbial contamination in sterile production lines during media fill tests.[3]
Peptones and glucose are sources of nitrogen, carbon, vitamins and trace elements needed for the growth of most non-fastidious and moderately fastidious microorganisms (bacteria, yeasts, fungi). Sodium chloride maintains osmotic balance, dipotassium hydrogen phosphate is included as a buffer system.

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 30 g in 1000 mL of cold purified sterile water. Mix thoroughly and warm slightly if necessary to completely dissolve the powder. Use according to the purpose required.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution appearance	yellow, limpid
Final pH at 20-25°C	7.3 ± 0.2
Gamma-irradiation	25 - 35 kGy

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptic Soy Broth γ-Irradiated	Dehydrated medium	402155G2 402155G4	500 g (16.7 L) Gamma-irradiated, triple bagged 5 kg (167 L) Gamma-irradiated, triple bagged

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Incubator and laboratory equipment as required.

8 - TEST PROCEDURE

Use the medium according to the purpose required. For “media fill” test[3] the culture medium is used in place of the product solution to test whether the aseptic procedures are adequate to prevent contamination during production process.
A media fill is one part of the validation of an aseptic manufacturing process.
After the final product container is filled and ready for release, it should be incubated in a temperature-controlled incubator. Any controlled temperature between 20 and 35° C would work for media fills. However, the “controlled temperature” should be specified in the procedures and be maintained within a range that does not exceed ± 2.5°C.
The incubation period of a media fill should be no less than 14 days and the containers should be examined every 2 or 3 days. If different incubation temperatures are chosen, it is recommended to incubate the containers filled with the medium for at least 7 days at the lowest temperature (e.g., 20- 25°C) and then 7 at the highest temperature (e.g., 30 - 35 ° C).

9 - READING AND INTERPRETATION

The presence of microorganisms is indicated by a varying degree of turbidity, specks and flocculation in the medium. The un-inoculated control remains clear and without turbidity after incubation. The characteristics of the growths are closely related to the type or types of microorganisms grown.

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. Here below are listed some test strains useful for the quality control.[2]

CONTROL STRAINS	INCUBATION T°/ t / ATM	EXPECTED RESULTS
C. albicans ATCC 10231	20-25°C / 24-72h -A	good growth

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it

A. brasiliensis ATCC 16404	20-25°C / 72-120h -A	good growth
B. subtilis ATCC 6633	30-35°C / 24 h -A	good growth
S. aureus ATCC 6538	30-35°C / 24 h -A	good growth
P. aeruginosa ATCC 9027	30-35°C / 24 h -A	good growth

A: aerobic incubation; 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 Tryptic Soy Broth γ-Irradiated, is tested for productivity by comparing the results with not-irradiated Tryptic Soy Broth.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes, incubating at 30-35°C or at 20-25°C for 18-24 hours or for 24-72 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: *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231, *A. brasiliensis* ATCC 16404, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 9027, *E. coli* ATCC 8739, *S.* Typhimurium ATCC 14028.
The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
The dehydrated medium is also subjected to bioburden evaluation before irradiation and to sterility test in compliance with the European Pharmacopoeia after irradiation.[2]

12 - LIMITATIONS OF THE METHOD

- Trptic Soy Broth γ-Irradiated is not suitable for the cultivation of fastidious microorganisms (e.g., *Haemophilus* or *Neisseria* spp.) and for the cultivation of strict anaerobes.
- Sub-cultures onto suitable solid media are necessary for purification of the culture and to perform identification tests.
- Due to the wide variety of production processes and devices to be examined with "media fill" test, it is the user's responsibility to validate this medium for the specific intended use.

13 - 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 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.
- 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 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 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.

14 - 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).

15 - REFERENCES

1. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
2. European Pharmacopoeia 11th Edition, 2022, Vol. 1.
3. FDA Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice

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 1	Updated layout and contents	2022/12

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

Biolife Italiana S.r.l., Viale Monza 272, 20128 Milan, Italy.
Tel. +39 02 25209.1, Fax +39 02 2576428
E-mail: mktg@biolifeitaliana.it; export@biolifeitaliana.it; web: www.biolifeitaliana.it