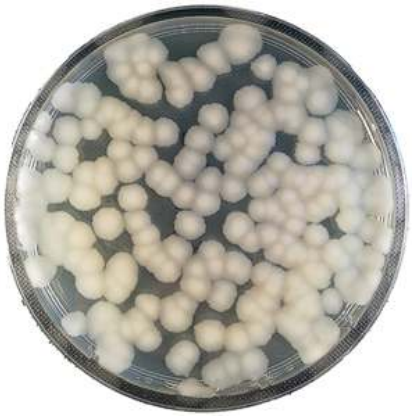
INSTRUCTIONS FOR USE

TRYPTIC SOY AGAR

Ready-to-use medium



Bacillus cereus on Tryptic Soy Agar

1 - INTENDED USE

In vitro diagnostic device. General purpose medium for cultivation, isolation and maintenance of non-fastidious and moderately fastidious microorganisms. For microbial enumeration of non-sterile pharmaceutical products and cosmetics.

2 - COMPOSITION -TYPICAL FORMULA *

Pancreatic digest of casein	15 g
Soy peptone	5 g
Sodium chloride	5 g
Agar	15 g
Purified water	1000 mL

*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 Agar (TSA) is one of the most widely used culture media in clinical and industrial microbiology. TSA has a multitude of uses in clinical and non-clinical laboratories including isolation, cultivation and purification of colonies of non-fastidious and moderately fastidious microorganisms and maintenance of stock cultures.[1] As it doesn't contain the X and V factors, it is suitable for identification of *Haemophilus* sp. by adding on the agar surface discs or strips impregnated with X (Hemin) and V (NAD) factors.[2] It is recommended as a reference medium, when testing selective media, to measure the degree of inhibition.[3] TSA is the medium specified as "casein soya bean digest agar" in the harmonised EP, USP JP method[4] for microbial enumeration of non-sterile pharmaceutical products. It is recommended by ISO Standard 21149 for the enumeration and detection of aerobic mesophilic bacteria in cosmetics[5].
Tryptic Soy Agar is prepared with selected casein and soy peptones: the combination of casein and soy peptones renders the medium nutritious by supplying organic nitrogen in the form of amino acids and polypeptides. Sodium chloride maintains the osmotic balance. Agar is the solidifying agent.

4 - PHYSICAL CHARACTERISTICS

Medium appearance	pale yellow, limpid
Final pH at 20-25 °C	7.3 ± 0.2

5 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Tryptic Soy Agar	Ready-to-use plates	542150	2 x 10 plates ø 90 mm primary packaging: 2 cellophane sachets secondary packaging: cardboard box

6 - MATERIALS REQUIRED BUT NOT PROVIDED

Sterile loops, needles and swabs, incubator and laboratory equipment as required, ancillary culture media and reagents for the identification of the colonies.

7 - SPECIMENS

Tryptic Soy Agar should not be used for the direct inoculation of clinical specimens. Generally, TSA is used for the sub-culture of microorganisms grown on other culture media. Non-clinical samples analyzed with Tryptic Soy Agar include non-sterile pharmaceutical products and cosmetics. Refer to the quoted literature for sample collection and preparation.[4,5]

8 - TEST PROCEDURE

Allow plates to come to room temperature and to dry the surface of the medium.
For the subculture of colonies, by means of a sterile needle or loop, inoculate an un-supplemented TSA plate with a colony cultivated on another isolation medium. The user is responsible for choosing the appropriate incubation time, temperature and atmosphere depending on the inoculated organism and the local applicable protocols.
For the microbial enumeration in non-sterile pharmaceutical products and cosmetics consult the references.[4,5]

9 - READING AND INTERPRETATION

After incubation, the presence of microorganisms is indicated by the appearance of colonies of various morphology and size on the un-supplemented medium surface. The characteristics of the growth are closely related to the type or types of cultivated microorganisms.

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 of un-supplemented medium.

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CONTROL STRAINS		INCUBATION T°/ t / ATM	EXPECTED RESULTS
S.aureus	ATCC 25923	35-37°C / 18-24H / A	good growth
E.coli	ATCC 25922	35-37°C / 18-24H / A	good growth

User quality control of TSA used for microbial enumeration in non-sterile pharmaceutical products and cosmetics should meet the requirements of EP[4] and ISO Standard[5]
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 ready to use plates of Tryptic Soy Agar and of the raw material used for the production of prepared plates, dehydrated Tryptic Soy Agar, (Test Batch: TB), are tested for productivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a quantitative test with the following strains: *P.aeruginosa* ATCC 9027, *E.coli* ATCC 8739, *B.cereus* ATCC 11778, *B.subtilis* ATCC 6633, *S.aureus* ATCC 6538, *S.aureus* ATCC 25923, *L.monocytogenes* ATCC 13932, *C.albicans* ATCC 10231, *A.brasiliensis* ATCC 16404. Tryptic Soy Agar plates are inoculated with decimal dilutions in saline of the colonies' suspensions and incubated at 30-35°C for 24-72 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr = CFU_{TB}/CFU_{RB}$) is calculated. If *Pr* is ≥ 0.7 and if the colonies morphology is typical, the results are considered acceptable and conform to the specifications.

12 - LIMITATIONS OF THE METHOD

- Even if the microbial colonies on the plates are differentiated on the basis of their morphological and chromatic characteristics, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.
- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

13 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- This product is not classified as dangerous according to current European legislation.
- 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 ready-to use plates 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.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- 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.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the in vitro diagnostic.
- 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 plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g. microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

15 - REFERENCES

1. Atlas R. Parks LC. Handbook of Microbiological Media. 2nd edition CRC Press,1997
2. Ledeboer NA, Doern GV. Haemophilus. In Jorgensen JH, Carrol KC, Funke G et al. editors. Manual of clinical microbiology,11th ed. Washington, DC: American Society for Microbiology; 2015. p.667.
3. ISO 11133:2014. Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media
4. European Pharmacopoeia, current edition
5. ISO 21149:2017. Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	For single use only	Fragile, handle with care

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REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2020/09
Revision 2	Removal of obsolete classification	2023/03

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

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