

# Trypticasein Soy Broth (TSB) EP/USP/ISO

Cat. 1224

For general laboratory use and to cultivate fastidious microorganisms.

## Practical information

Applications	Categories
Enrichment	General use
Selective enrichment	Bacillus cereus
Diluent	General use

Industry: Pharmaceutical/Veterinary / Cosmetics / Clinical / Food

Regulations: USP / ISO 10273 / ISO 11133 / European Pharmacopoeia / ISO 21871



## Principles and uses

Trypticasein Soy Broth (TSB) is a medium very rich in nutrients for general use in microbiological laboratories. It supports the abundant growth of fastidious organisms such as pneumococci, streptococci, Neisseriae, etc.

The medium is used frequently in many procedures of diagnostic research or microbiology. For example, it is used for the isolation and sensitivity testing of all types of pathogens, and for the production of antigens for agglutination and serological tests.

Containing two peptones as rich nitrogen sources obtained by the enzymatic hydrolysis of casein and soy proteins. This medium supports the growth of a great variety of microorganisms, including fastidious aerobes and anaerobes. Soy peptone also contains natural sugars which promote bacterial growth. Glucose is a carbohydrate and carbon source. Sodium chloride supplies essential electrolytes for transport and osmotic balance, and dipotassium hydrogen phosphate is a buffering agent.

ISO 21871 recommends the addition of a polymyxin solution for the enumeration of low numbers of viable presumptive *Bacillus cereus* (TSPB medium).

The ISO 10273 standard recommends this medium to preserve positive strains of pathogenic *Yersinia* as frozen reserve cultures.

The European Pharmacopoeia, USP recommends this medium in the paragraph 2.6.12: "Microbiological examination of non – sterile products: Microbial enumeration test" for the preparation of the samples for the examination of TAMC and TYMC in products, and in the paragraph 2.6.13 "Microbiological examination of non-sterile products: Test for specified microorganisms" for the preparation of samples for de examination of specified microorganism. Also, in the paragraph 2.6.1: "Microbiological examination of Sterile products" for the test for sterility of both fungi and aerobic bacteria.

If desired, antibiotics can easily be incorporated as well as other supplements or inhibitory agents.

## Formula in g/L

Glucose monohydrate	2,5	Pancreatic digest of casein	17
Sodium chloride	5	Papaic digest of soyabean meal	3
Dipotassium hydrogen phosphate	2,5		

## Preparation

Suspend 30 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121 °C for 15 minutes. Larger quantities may require a longer sterilization time, but the temperature should not be increased.

## Instructions for use

» For clinical diagnosis, the type of samples is bacterial strains isolated from other media.

- Inoculate the medium with the desired strain of 10-100 CFU.
- Incubate in aerobic conditions at 35±2 °C for 18-72 hours.
- Reading and interpretation of the results.

» For other uses not covered by the CE marking:

Enumeration of low numbers of viable presumptive *Bacillus cereus* (TSPB medium) according to ISO 21871:

- Dispense in tubes in quantities of 10 ml from double concentration medium or 9 ml from simple concentration medium.
- Immediately before its use, add either 200 µl (double concentration medium) or 100 µl (simple concentration medium) from the solution of polymyxin 500000 UI (approximately 0,05 gr /50 ml sterile water).

Examination of TAMC and TYMC in products according to European Pharmacopoeia:

Membrane filtration:

- Prepare the product sample suspending, dissolving or diluting the product to be examined in the Trypticasein Soy Broth.
- Transfer the appropriate amount of the sample to a membrane filter.
- Place the membrane to the surface of Trypticasein Soy Agar (Cat. 1068) in case of TAMC or Sabouraud Dextrose Agar (Cat. 1024) in case of TYMC.
- Incubate the plate of Trypticasein Soy Agar (Cat. 1068) at 30-35 °C for 3-5 days and the plate of Sabouraud Dextrose Agar (Cat. 1024) at 20-25 °C for 5-7 days.

Plate-count methods:

- Prepare the product sample suspending, dissolving or diluting the product to be examined in the Trypticasein Soy Broth.
- Inoculate the plates of Trypticasein Soy Agar (Cat. 1068) in case of TAMC or Sabouraud Dextrose Agar (Cat. 1024) in case of TYMC, conforming to the pour-plate method or the surface-spread method.
- Incubate the plates of Trypticasein Soy Agar (Cat. 1068) at 30-35 °C for 3-5 days and the plates of Sabouraud Dextrose Agar (Cat. 1024) at 20-25 °C for 5-7 days.
- Select the plates corresponding to a given dilution and showing the highest number of colonies less than 250 (TAMC) or 50 (TYMC).

Most-probable number method (only for TAMC):

- Prepare and dilute the product sample to be examined, and inoculate into tubes of Trypticasein Soy Broth.
- Incubate all tubes at 30-35 °C for 3-5 days.
- Record for each level of dilution the number of tubes that showing growth and determinate the most probable number of microorganisms.

Sterility test for fungi and aerobic bacteria according to European Pharmacopoeia:

- Prepare the product to be examined.
- Transfer the preparation to a membrane filter and add the membrane to the Trypticasein Soy Broth, or inoculate directly the appropriate quantity of the preparation into the Trypticasein Soy Broth (the volume of the product no more than 10% of the volume of the medium).
- Incubate the medium at a temperature of 20-25 °C not less than 14 days.
- If no growth of microorganisms occurs, the product is sterile.

## Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Amber	7,3 ± 0,2

## Microbiological test

According to European Pharmacopoeia, USP: Total aerobic microbial count:

Incubation conditions: (30-35 °C / <=3 days: bacteria / <=5 days: fungi ).

Inoculation conditions: (<= 100 CFU).

According to ISO 11133 TSPB:

Incubation conditions: ( 30±1 °C/ 48±4 h).

Inoculation conditions: Productivity qualitative (<=100 CFU) / Selectivity (10<sup>4</sup>-10<sup>6</sup> CFU).

Microorganisms	Specification	Characteristic reaction
<i>Bacillus cereus</i> ATCC 11778	Turbidity on TSPB. > 10 colonies on PEMBA or MYP	Turquoise blue colonies with precipitation halo on PEMBA. Pink colonies with precipitation halo on MYP.
<i>Escherichia coli</i> ATCC 25922	Total inhibition on TSPB. Total inhibition (0) on TSA.	
<i>Staphylococcus aureus</i> ATCC 6538	Good growth, turbidity	
<i>Bacillus subtilis</i> ATCC 6633	Good growth, turbidity.	
<i>Pseudomonas aeruginosa</i> ATCC 9027	Good growth, turbidity.	

## Storage

Temp. Min.: 2 °C

Temp. Max.: 25 °C

## Bibliography

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Harmonized European Pharmacopoeia

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ISO 21871:2006. *Microbiology of food and animal feeding stuffs -- Horizontal method for the determination of low numbers of presumptive Bacillus cereus -- Most probable number technique and detection method*

ISO 10273:2017. *Microbiology of the food chain -- Horizontal method for the detection of pathogenic Yersinia enterocolitica*

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