



TRYPTICASEIN SOY BROTH (TSB) EUROPEAN PHARMACOPOEIA

CAT Nº: 1224 For general laboratory use and to cultivate fastidious microorganisms

FORMULA IN g/l

| Final pH 7.3 ± 0.2 at 25°C | | | | | | |
|-----------------------------|-------|-----------------------|------|--|--|--|
| Papaic Digest of Soy Bean | 3.00 | | | | | |
| Sodium Chloride | 5.00 | Dipotassium Phosphate | 2.50 | | | |
| Pancreatic Digest of Casein | 17.00 | Glucose Monohydrate | 2.50 | | | |

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. The prepared medium should be stored at 2-8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

TRYPTICASEIN SOY BROTH 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 phosphate is a buffering agent.

The European Pharmacopoeia recommends this medium in Paragraph 2.6.13 "Microbiological examination of non-Sterile products" for the test of specified microorganisms, as a pre-enrichment medium for sample preparations for testing of products. Inoculate and incubate at 30-35°C and observe after 18 - 24 hours.

In Paragraph 2.6.12 "Microbiological examination of non – sterile products: Microbial enumeration test", it is indicated to inoculate and incubate at $30-35^{\circ}C \le 3$ days for the total count of aerobics ≤ 100 cfu/ml.

The European Pharmacopoeia also recommends this medium in Paragraph 2.6.1. "Microbiological examination of Sterile products" for the culture of both fungi and aerobic bacteria. It recommends inoculating portions of soya bean casein digest medium with a small number (not more than 100 UFC) of the following micro-organisms, using a separate portion of medium for each of the following species of micro-organisms: *Aspergillus brasiliensis, Bacillus subtilis, Candida albicans.* Incubate for not more than 3 days in the case of bacteria and not more than 5 days in the case of fungi. It is indicated to be incubated at 20-25 °C.

For a growth promotion test of aerobes, anaerobes and fungi, inoculate the medium with a small number of microorganisms: no more than 100 cfu of *Aspergillus brasiliensis* ATCC 16404, *Bacillus subtilis* ATCC 6633 and *Candida*





albicans ATCC 10231. Incubate for no more than 3 days in the case of bacteria, and no more than 5 days in the case of fungi.

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

Sterility Test: Incubate portions of the media for 14 days. If no growth of microorganisms occurs, the product is sterile.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30-35°C and observed after 18-24 hours.

| Microorganisms | Growth | Inoculum cfu | Recovery |
|--|--------|----------------------------------|----------|
| Brucella abortus ATCC 4315 | Good | 10 ² -10 ³ | ≥50 |
| * Staphylococcus aureus ATCC 6538 | Good | ≤100 | ≥70 |
| Escherichia coli ATCC 25922 | Good | $10^2 - 10^3$ | ≥70 |
| Escherichia coli ATCC 8739 | Good | 10 ² -10 ³ | ≥70 |
| Enterobacter aerogenes ATCC 13048 | Good | $10^2 - 10^3$ | ≥70 |
| ** Candida albicans ATCC 10231 | Good | ≤100 | ≥70 |
| Streptococcus pyogenes ATCC 19615 | Good | $10^2 - 10^3$ | ≥70 |
| Streptococcus pneumoniae ATCC 6303 | Good | 10 ² -10 ³ | ≥70 |
| * Pseudomonas aeruginosa ATCC 9027 | Good | ≤100 | ≥70 |
| Salmonella typhimurium ATCC 14028 | Good | 10 ² -10 ³ | ≥70 |
| * Bacillus subtilis ATCC 6633 | Good | ≤100 | ≥70 |
| ** Aspergillus brasiliensis ATCC 16404 | Good | ≤100 | ≥70 |

^{*}According to European Pharmacopeia. Total recount of aerobiosis ≤100 cfu/ml. Incubate at 30-35°C for ≤3 days.

BIBLIOGRAPHY

European Pharmacopoeia.7.0

Gibbons and McDonald. J. Bacteriol., 80:164. 1960. Havens and Benham. A. Med. Tech., 23:305. 1957.

Muey and Edward. Proc. Soc. Exper. Biol. and Med., 97:550. 1958. Steward and Kelly. J. Bacteriol., 77:101. 1959.

MacFaddin, J.D. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 797. vol. 1. Williams & Wilkins, Baltimore, MD.



STORAGE

Once opened keep powdered medium closed to avoid hydration.







^{**} According to European Pharmacopeia. Total recount of yeast and molds ≤100 cfu/ml. Incubate at 30-35°C for ≤5 days.