



## Tryptic Soy Broth + 15% Glycerol

Liquid medium for the preservation of organisms by freezing.

### INTENDED PURPOSE

Tryptic Soy Broth + 15% Glycerol is a liquid medium used in the long-term frozen storage of microorganisms.

### DESCRIPTION

Tryptic Soy Broth (TSB) or Casein Soya Bean Digest Broth is a highly nutritious medium used for the cultivation of a wide range of microorganisms. It is recommended by the harmonized USP/EP/JP method for sterility testing and for microbiological examination of non-sterile products. TSB supplemented with 15% glycerol (v/v) is used to maintain bacterial viability for extended periods by freezing.

### TYPICAL FORMULA\* (Per Liter of Purified Water)

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soya Bean	3.0 g
Sodium Chloride	5.0 g
Dipotassium Hydrogen Phosphate	2.5 g
Glucose Monohydrate	2.5 g
Glycerol	150.0 ml
Final pH 7.3 ± 0.2 at 25°C	

\*Adjusted and/or supplemented as required to meet performance specifications.

### METHOD PRINCIPLE

Enzymatic digest of casein and soya bean provide amino acids, nitrogen, carbon, vitamins and minerals for organisms growth. Sodium chloride maintains osmotic balance in the medium. Dipotassium phosphate is a buffering agent. Glucose is an energy source. Glycerol acts as a cryoprotectant to reduce cell damage caused by the freezing process.

### MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as: sterile loops/swabs, solid culture media, incubator, freezer, quality control organisms.

### TEST PROCEDURE

1. Prepare an overnight culture of the organism to be stored on an appropriate non-selective solid medium, such as Tryptic Soy Agar (TSA) or Blood agar.
2. Using a sterile swab or inoculating loop, remove fresh growth from the culture medium and suspend the colonies in the tube to achieve the desired concentration of viable cells.
3. Shake or vortex well before freezing suspension at -20°C or below.

### Recovery

1. Allow the tube to thaw by placing it at room temperature.
2. Under aseptic conditions open the tube and use the contents to inoculate a suitable broth or agar medium.

### INTERPRETING RESULTS

After incubation observe the growth indicating the presence of a viable organism from the frozen culture.

### STORAGE

Store at 10-25°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

### SHELF LIFE

- 1 year for plastic tubes.
- 2 years for glass tubes.

## QUALITY CONTROL

**Appearance of medium:** Clear with none to light precipitate, yellow to amber.

### Expected Cultural Response:

The inoculated tubes are frozen at  $-20^{\circ}\text{C}$  for 48 hours and sub-cultured onto Tryptic Soy Agar.

Control strains	Incubation	Specification
<i>Escherichia coli</i> ATCC® 25922	24-48 h/ $35 \pm 2^{\circ}\text{C}$	Good growth on TSA
<i>Pseudomonas aeruginosa</i> ATCC® 27853		Good growth on TSA
<i>Staphylococcus aureus</i> ATCC® 25923		Good growth on TSA

Please refer to the actual batch related Certificate of Analysis (CoA).

## PERFORMANCE CHARACTERISTICS

Performance testing of Tryptic Soy Broth + 15% Glycerol was carried out using the QC strains listed above. The results obtained met the established criteria.

## LIMITATIONS

Invalid results can be caused by poor sample quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

The specific length of time that a culture will remain viable in a given storage condition is dependent upon the bacterial strain.

Repeated thawing and refreezing of the bacterial stocks should be avoided due to decreased organism viability and possible contamination.

Initial cell density influences the recovery after thawing. For most bacteria, a density of  $10^7$  cells/ml will result in adequate recovery if all conditions are properly maintained.

## WARNING AND PRECAUTIONS

**For professional use only.** Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

## DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

## BIBLIOGRAPHY

See the references at the end of this document.

## TABLE OF SYMBOLS

See the table of symbols at the end of this document.

**ORDER INFORMATION**

Product	Format	Packaging	Ref.
Tryptic Soy Broth + 15% Glycerol	10x47mm Plastic Tube with screw cap and self-standing base	20 x 1.5 ml	27002
	20x100mm Tube	20 x 7 ml	24445

**Revision History**

Revision	Release Date	Change Summary
0	2025-08-04	Updated layout and content, version reset to revision 0

This IFU document and the SDS are available from the online Support Center:

[liofilchem.com/ifu-sds](https://liofilchem.com/ifu-sds)

## References

1. United States Pharmacopeial Convention (2014) The United States Pharmacopeia 38/National Formulation 33, Supp. 2. Chapter <61> Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter <62> Microbiological examination of non-sterile products: Test for specified products. Chapter <71> Sterility Tests. Rockville, Md., USA.
2. European Directorate for the Quality of Medicines and Healthcare (2014) The European Pharmacopoeia. 8th Ed. Chapter 2.6.12 Microbiological examination of non-sterile products: Microbial enumeration tests and Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.
3. Japanese Ministry of Health, Labour and Welfare (2011) The Japanese Pharmacopoeia. 16th Ed. Chapter 4.05 Microbial Limit Test I. Microbiological examination of non-sterile products: Total viable aerobic count and II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.
4. CLSI. Quality Assurance for Commercially Prepared Microbiological Culture Media, Approved Standard - 3rd ed. CLSI document M22-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.
5. Gherna, RL (1994) Culture preservation, p. 278-292. In P. Gerhardt, R.G.E. Murray, W.A. Wood, and N.R. Krieg (ed.) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, D.C.
6. MacFaddin, JF (1985) Media for Isolation, Cultivation, Identification, Maintenance of Bacteria, Vol. I. Williams & Wilkins, Baltimore, MD.
7. Kirsop, BE and JJS Snell (ed.) (1984) Maintenance of microorganisms. Academic Press Inc., New York.

## Table of Symbols

	Batch code
	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult instructions for use
	Do not reuse
	Keep away from sunlight



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