

Technical Data Sheet

Tryptic Soy Broth + Neutralizers

Ordering number: 1.46492.0010

Tryptic Soy Broth with neutralizers is a universal complex medium for the isolation and cultivation of fastidious aerobic bacteria, yeasts and molds in the presence of disinfectant residues.

The medium is designed for the testing of sterile and non-sterile substances, preparations and products according to the European Pharmacopoeia (EP) and the United States Pharmacopoeia (USP).

The formulation of the basic medium (Soybean-Casein Digest Broth) is prepared according to the recommendations of the current European, Japanese and United States Pharmacopoeia (EP, 2.6.1.; JP, 4.06 and USP, 71).

Tryptic Soy Broth with neutralizers is available in various formulations and filling volumes and with various closure types:

- Tryptic Soy Broth + LT (article number 146484): 250 ml-bottle with **screw cap**, filling volume 90 ml
- Tryptic Soy Broth + LTH (article number 146268): 17 ml-**tube**, filling volume 10 ml
- Tryptic Soy Broth + LTH (article number 146440): 275 ml-bottle with **wide mouth**, filling volume 90 ml
- Tryptic Soy Broth +TLH (article number 146493): 1000 ml-bottle with **screw cap**, filling volume 1000 ml
- Tryptic Soy Broth + LTHTh (article number 146283): 17 ml-**tube**, filling volume 10 ml
- Tryptic Soy Broth + LTHTh (article number 146485): 275 ml-bottle with **wide mouth**, filling volume 90 ml
- Tryptic Soy Broth + TLHC (article number 146492): 125 ml-bottle with **screw cap**, filling volume 90 ml
- Tryptic Soy Broth + TLHC (article number 146640): 250 ml-bottle with **screw cap**, filling volume 250 ml

Mode of Action

Tryptic Soy Broth is a highly nutritious media and hence supports the growth of a wide variety of aerobic and facultative anaerobic microorganisms including fungi.

The neutralizing additives Polysorbate (Tween®) 80, Na-thiosulfate, cysteine, histidine and lecithin reverse the growth inhibitory effect of most disinfectants and antiseptics. Neutralizing efficiency of

histidine could be shown against formaldehyde and formaldehyde releasing agents. Na-thiosulfate inactivates sodium hypochlorite and acidified sodium chlorite. Cysteine is a reducing agent, neutralizing oxidizing agents such as hydrogen peroxide and iodine. Lecithin, particularly in combination with Polysorbate 80, is effective against quaternary ammonium compounds, amphoteric surfactants, benzamidines, chlorhexidines and dequadin. Polysorbate 80 inactivates benzyl alcohol, carbanilides, dichlorobenzyl alcohols, benzoic acid, p-hydroxybenzoic acid and its esters, phenols, phenylethyl alcohols and Solbrols (PHB esters).

Typical Composition

	Art. No. 146492	Other Products			
Additive	TSB + TLHC	TSB + LT	TSB + LTH	TSB + LTHTh Art. No. 146485	TSB + LTHTh Art. No. 146283
Casein Peptone	17 g/l	17 g/l	17 g/l	17 g/l	17 g/l
Soy Peptone	3 g/l	3 g/l	3 g/l	3 g/l	3 g/l
NaCl	5 g/l	5 g/l	5 g/l	5 g/l	5 g/l
Phosphate Buffer	2.5 g/l	2.5 g/l	2.5 g/l	2.5 g/l	2.5 g/l
Glucose Monohydrate	2.5 g/l	2.5 g/l	2.5 g/l	2.5 g/l	2.5 g/l
Lecithin	3 g/l	0.7 g/l	3 g/l	3 g/l	3 g/l
Polysorb. (Tween®) 80	30 ml/l	5 ml/l	30 ml/l	30 ml/l	30 ml/l
Histidine	1 g/l	-	1 g/l	1 g/l	1 g/l
Cysteine	1 g/l	-	-	-	-
Sodium Thiosulfate	-	-	-	0.1 g/l	5 g/l
Appearance	clear to slightly turbid, yellowish	clear, yellow	clear, yellowish	clear, yellowish	clear, yellowish to yellow (sediment of neutralizers & fine particles possible)

The pH value is in the range of 7.1-7.5. The media can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

Tryptic Soy Broth with Neutralizers is provided in tube and bottle and can be used for the detection of aerobic and anaerobic microorganisms in sterile and non-sterile products.

Sterility testing:

According to the European and US Pharmacopoeia, sterility test may be carried out using the technique of membrane filtration or by direct inoculation.

For membrane filtration, diluents e.g. Fluid A (article number 146470) or Fluid D (article number 146397) may be used. The filter or its half is transferred into Tryptic Soy broth with Neutralizers or vice versa and incubated at 20-25 °C for not less than 14 days.

For direct inoculation the volume of the sample which has to be tested should not extend 10 % of the volume of the Tryptic Soy Broth with Neutralizers and incubated at 20-25 °C for not less than 14 days.



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If the material to be tested renders the medium turbid and a visual examination is not possible after 14 days of incubation, portions not less than 1 ml of the inoculated Tryptic Soy Broth with Neutralizers are transferred into fresh Tryptic Soy Broth with Neutralizers. The original inoculated broth as well as the freshly inoculated broth are incubated at 20-25 °C for not less than 4 days and examined again.

In case of growth it is recommended to identify the colonies using microbiological methods (e.g. selective media, biochemical methods) in order to identify and remove the source of contamination.

Non sterile testing:

For total aerobic microbial count (TAMC), the product to be tested should be prepared as described in EP (2.6.12). 1g or 1ml of the prepared sample is used to inoculate Tryptic Soy Broth with Neutralizers, which is then incubated at 30-35°C for up to 3 days. Subculture can be performed on Tryptic Soy Agar (Article No: 146004) and incubated at 30-35°C for 24-48 h.

Storage and Shelf Life

The product can be used for tests until the expiry date if protected from light and properly sealed at +2 °C to +25 °C.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Result
<i>Staphylococcus aureus</i>	6538	10-100	24-48 h at 33-35 °C	good growth; pronounced turbidity
<i>Escherichia coli</i>	8739	10-100	24-48 h at 33-35 °C	good growth; pronounced turbidity
<i>Pseudomonas aeruginosa</i>	9027	10-100	24-48 h at 33-35 °C	good growth; pronounced turbidity
<i>Bacillus subtilis</i>	6633	10-100	24-48 h at 33-35 °C	good growth; pronounced turbidity
<i>Candida albicans</i>	10231	10-100	48-74 h at 20-25 °C	good growth; pronounced turbidity
<i>Aspergillus brasiliensis</i>	16404	10-100	48-74 h at 20-25 °C	good growth; pronounced flocculation
<i>Escherichia coli</i> + 200µl Aerodesin 2000	8739	10-100	24-48 h at 33-35 °C	good growth; pronounced turbidity

For incubation specification, please refer to the actual batch related Certificate of Analysis.



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Literature

Brown, M.R.W. (1966): Turbidimetric method for the rapid evaluation of antimicrobial agents. Inactivation of preservatives by nonionic agent. – J. Soc. Cosm. Chem., 17; 185-195.

European Pharmacopoeia 8.0 (2014): 2.6.1. Sterility; 2.6.12. Microbial examination of non-sterile products (total viable aerobic count);

Holdeman, L. V., Cato, E.P., Moore, W.E.C (1977): Anaerobe Laboratory Manual. Virginia Polytechnic Institute and State University. Clacksburg, VA.

HUGO, W.B., a. FRIER, M.: Mode of action on the antibacterial compound desqualinium acetate. – Appl. Microbiol., 17; 118-127 (1969).

Japanese Pharmacopoeia 16th edition (2011): 4.05 Microbial Limit Test; 4.06 Sterility Test.

MacFaddin, J.F. 1985. Media for isolation-cultivation- identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.

Russel, A.D., Ahonkhai, I. and Rogers, D.T. (1979) Microbiological applications of the inactivation of antibiotics and other antimicrobial agents. *Journal of Applied Bacteriology* 46:207-245.

United States Pharmacopoeia 38 NF 33 (2015): <71> Sterility Tests; <61> Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests.

Ordering Information

Product	Cat. No.	Pack size
Tryptic Soy Broth + TLHC	1.46492.0010	10 x 90 ml bottle
Tryptic Soy Broth + LT	1.46484.0006	6 x 90 ml bottle
Tryptic Soy Broth + LTH	1.46268.0100	100 x 10 ml tube
Tryptic Soy Broth + LTH	1.46440.0006	6 x 90 ml bottle
Tryptic Soy Broth + TLH	1.46493.0006	6 x 1000 ml bottle
Tryptic Soy Broth + LTHTh	1.46283.0020	20 x 10 ml tube
Tryptic Soy Broth + LTHTh	1.46283.0100	100 x 10 ml tube
Tryptic Soy Broth + LTHTh	1.46485.0006	6 x 90 ml bottle
Tryptic Soy Broth + TLHC	1.46640.0006	6 x 250 ml bottle
Fluid A	1.46470.0010	10 x 100 ml bottle
Fluid D	1.46397.0010	10 x 100 ml bottle
Tryptic Soy Agar - LI	1.46004.0020	20 x 90 mm plate
Tryptic Soy Agar - LI	1.46004.0120	120 x 90 mm plate

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