Technical Data Sheet

Fluid Thioglycollate Medium + LTH acc. EP + USP

Ordering number: 1.46679.0006

Fluid Thioglycollate Medium (FTM) with LTH is a universal complex medium for the isolation and cultivation of fastidious anaerobic as well as for aerobic microorganisms. It is used for sterility testing of pharmaceutical products in the presence of disinfectant residues.

The medium is used for sterility testing of substances, preparations and products according to European Pharmacopoeia (EP) and the United States Pharmacopoeia (USP).

The formulation of the basic medium (Fluid Thioglycollate Medium) is prepared according to the recommendations of the current European and United States Pharmacopoeia (EP, 2.6.1. and USP, 71).

Fluid Thioglycollate Medium + LTH is available in 500 ml-bottle with combined **septum and screw cap**, filling volume 500 ml.

Mode of Action

Thioglycollate and L-Cystine in the medium reduce the redox potential of the culture medium in order to create an anaerobic atmosphere. In addition, mercury and other heavy metal compounds are inactivated by these agents. The content of agar further reduces a rapid diffusion of oxygen through the medium, but may lead to a slight turbidity in larger volumes (filled bottles). Resazurin indicates the reduction potential of the medium. An increased concentration of oxygen is indicated by a color change to pink. The neutralizing additives lecithin, Polysorbate (Tween®) 80 and histidine reverse the growth inhibitory effect of most disinfectants and antiseptics. Neutralizing efficiency of histidine could be shown against formaldehyde and formaldehyde releasing agents. Lecithin, particularly in combination with Tween® 80, is effective against quaternary ammonium compounds, amphoteric surfactants, benzamidines, chlorhexidines and dequadin. Tween® 80 inactivates benzyl alcohol, carbanilides, dichlorbenzyl alcohols, benzoic acid, p-hydroxybenzoic acid and its esters, phenols, phenylethyl alcohols and Solbrols (PHB esters).

Typical Composition

Casein Peptone	15 g/l
Yeast Extract	5 g/l
Na-Thioglycollate	0.5 g/l
Resazurin	1 mg/l
L-Cystine	0.5 g/l
Glucose Anhydrate	5 g/l
NaCl	2.5 g/l
Soya Lecithine	5 ml/l
Polysorbate (Tween®) 20	40 ml/l
Histidine	1.6 ml/l
Agar	0.75 g/l

The appearance of the medium is turbid and yellowish. The pH value is in the range of 6.9-7.3. The medium can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

The surface of the containers is not sterile. Therefore, please be aware about a risk of secondary contamination due to handling. In order to reduce the risk of secondary contamination by defect glass containers or handling the following recommendations may be helpful:

- Please control each single container for visible defects or turbidity. Do not use such containers.
- Please avoid the contamination of culture media by contact with skin or body fluids. Such contaminated media cannot be used anymore.
- In the case of negative pressure due to prior heat-sterilization, the containers should be ventilated by sterile filter-units before usage in order to avoid aspiration of potential contaminated air.
- The risk of transfer of microorganisms from the surface of the containers into the sterile culture medium can be minimized by disinfection of these surfaces followed by handling in sterile environments, e.g. isolators. The inoculation of the containers or the media transfer into membrane filtration units by sterile cannulas is safer than procedures which require opening of media bottles or tubes.

Media which contain ingredients of animal or human origin such as blood, meat extract or animal tissues have to be considered potentially infectious. After contact of such media a disinfection of the affected skin area is recommended.

Strictly anaerobic microorganisms such as *Clostridium sporogenes* are growing in the lower, yellowish part of the broth medium. The growth of facultative anaerobic microorganisms such as *Staphylococcus aureus* is distributed in the complete broth medium. Aerobic microorganisms such as *Pseudomonas aeruginosa* are able to grow in the upper, oxidized part of Fluid Thioglycollate Medium indicated by a slight pink color. Usually the incubation is performed under aerobic conditions. In order to provide sufficient oxygen for the growth of aerobic microorganisms in bottles, which are filled under vacuum, a sterile ventilation device may be necessary to provide sufficient oxygen for the growth of aerobic microorganisms. But not more than the upper half of the medium should have been undergone a color change to pink indicative of oxygen uptake at the end of the incubation period.

Fluid Thioglycollate Medium is recommended for sterility testing of pharmaceutical products according to the European and US Pharmacopoeia. According to the Pharmacopoeia a membrane filtration method should be performed wherever possible, but also direct inoculation methods are possible.

For direct inoculation the amount of the inoculated sample material should not exceed 10 % of the volume of Fluid Thioglycollate Medium. Fluid Thioglycollate Medium is incubated for 14 days at 30 to 35°C and visually inspected for growth.

The sterility test is passed, if no growth is visible at the end of incubation.

It is recommended to identify grown microorganisms in order to find out the origin of contamination and to implement corrective actions.

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 Results
Clostridium sporogenes	11437	10-100	20-24 h at 30-35 °C	good growth; pronounced turbidity
	19404	10-100	20-24 h at 30-35 °C	good growth; pronounced turbidity
Pseudomonas aeruginosa	9027	10-100	20-24 h at 30-35 °C	good growth; pronounced
Staphylococcus aureus	6538	10-100	20-24 h at 30-35 °C	good growth; pronounced

Please refer to the actual batch related Certificate of Analysis.

Literature

American Public Health Association (2015), Compendium of Methods for the Microbiological Examination of Foods, 5th ed., APHA, Washington, D.C.

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.

Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice.

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

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 (2015): <71> Sterility Tests.

Ordering Information

Product	Cat. No.	Pack size
Fluid Thioglycollate Medium + LTH acc. EP + USP	1.46679.0006	6 x 500 ml bottle