# Alternative Thioglycollate Medium

**MAP010**

## (MU010/MM010)

## Intended Use:

Recommended for sterility testing of turbid or viscous biological products in accordance with USP/IP.

## Composition**

| Ingredients | g / L |
|---|---|
| Tryptone # | 15.000 |
| Yeast extract | 5.000 |
| Dextrose monohydrate | 5.500 |
| Sodium chloride | 2.500 |
| L-Cystine | 0.500 |
| Sodium thioglycollate | 0.500 |
| pH after sterilization | 7.1±0.2 |

**Formula adjusted, standardized to suit performance parameters

# Pancreatic digest of casein

## Directions

Suspend 28.5 grams (the equivalent weight of of dehydrated medium per litre) in 1000 ml purified/ distilled water. Heat if necessary to dissolve the medium completely. Distribute into flasks or tubes as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

*Note: It is preferable to use freshly prepared medium, alternatively it should be boiled and cooled just once prior to use or with reheating, toxic oxygen radicals are formed.*

## Principle And Interpretation

Alternative Thioglycollate Medium is formulated as described in N.I.H. Memorandum (1), U.S. Pharmacopoeia (2) and Indian pharmacopoeia (3). This medium is recommended for sterility testing for detecting the presence of viable forms of microorganisms in or on pharmaceutical preparations. This medium is also used for sterility checking for devices having tubes with small lumina. Alternative thioglycollate Medium is generally used for products containing mercurial preservatives when the oxidation-reduction indicator is not present or required. Lack of an indicator in the medium avoids possible toxicity to organisms.

Alternative Thioglycollate Medium contains sodium thioglycollate that can neutralize the bacteriostatic effect of mercurial preservatives. Absence of agar makes it suitable for testing viscous materials and devices having tubes with small lumina. Tryptone, yeast extract, dextrose monohydrate, L-cystine provides nitrogenous and carbonaceous compounds, vitamin B complex, trace elements and other essential growth nutrients. Sodium thioglycollate and L-cystine lower the oxidation-reduction potential of the medium by removing oxygen radicals and thus preventing the accumulation of peroxides that can be toxic to some organisms. The sulfhydryl groups of these compounds also neutralize the antibacterial effect of mercurial preservatives with heavy metals. Dextrose monohydrate is the fermentable carbohydrate energy source. Sodium chloride maintains the osmotic balance of the medium.

## Type of specimen

Pharmaceutical: Sterility testing of viscous products.

## Specimen Collection and Handling

For pharmaceutical products, follow appropriate techniques for sample processing in case of viscous materials as mentioned under sterility (2,3).

After use, contaminated materials must be sterilized by autoclaving before discarding.

## Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

## Limitations

1.The tubes should not be reheated as frequent boiling leads to development of toxic products.

2.Prior to use the medium should be boiled once to remove the absorbed oxygen.

3.Before inoculation, the tubes should be brought to room temperature

4. The medium should not be used in fermentation process as medium contains yeast extract which is high in carbohydrate content.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

**Appearance**

Cream to yellow homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Light yellow coloured clear solution without any precipitate.

**pH**

7.1±0.2

**Cultural Response**

Growth Promotion observed in accordance with USP/IP, under anaerobic condition after an incubation at 30-35°C for <=3 days.

| Organism | Inoculum (CFU) | Growth |
|---|---|---|
| **Growth Promotion Test** | | |
| *Clostridium sporogenes* ATCC 19404 | 50 -100 | luxuriant |
| *Clostridium sporogenes* ATCC 11437 | 50 -100 | luxuriant |
| ** *Phocaeicola vulgatus* ATCC 8482 | 50 -100 | luxuriant |
| **Additional Microbiological testing** | | |
| *Staphylococcus aureus* subsp. *aureus* ATCC 25923 (00034*) | 50 -100 | luxuriant |
| *Staphylococcus aureus* subsp. *aureus*ATCC 6538 (00032*) | 50 -100 | luxuriant |
| *Pseudomonas aeruginosa* ATCC 27853 (00025*) | 50 -100 | luxuriant |
| ^*Pseudomonas paraeruginosa* ATCC 9027 (00026*) | 50 -100 | luxuriant |
| *Escherichia coli* ATCC 25922 (00013*) | 50 -100 | luxuriant |
| *Escherichia coli* ATCC 8739 (00012*) | 50 -100 | luxuriant |
| *Salmonella* Abony NCTC 6017 (00012*) | 50 -100 | luxuriant |
| *Clostridium perfringens* ATCC 13124 (00007*) | 50 -100 | luxuriant |

| | | |
|---|---|---|
| *Bacteroides fragilis* ATCC 23745 | 50 -100 | luxuriant |

Key : *Corresponding WDCM numbers.
^ Formerly known as *Pseudomonas aeruginosa* **Formerly known as *Bacteroides vulgatus*

## Storage and Shelf Life

Store between 10-30°C in tightly closed container and the prepared medium at 15-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

## Reference

1. N.I.H. Memorandum, 1955 : Culture Media for Sterility Tests, 4th Revision.
2. The United States Pharmacopoeia-National Formulatory (USP-NF), 2022.
3. Indian Pharmacopoeia, 2022, Indian Pharmacopoeia Commission, Ministry of Health and Family Welfare Government of India.
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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