# Alternative Thioglycollate Medium

## Intended Use
Alternative Thioglycollate Medium is recommended for sterility testing of certain biological products, which may be turbid or viscous.

## Summary
Alternative Thioglycollate Medium is formulated as described in the N.I.H. memorandum. This medium is used for the sterility testing of certain biological products, which are turbid or viscous and cannot be tested using Fluid Thioglycollate Medium. Both the above media have similar composition, excluding agar and resazurin that are not included in Alternative Thioglycollate Medium. This deletion makes it appropriate for sterility testing of viscous products. For Alternative Thioglycollate Medium, anaerobic incubation is recommended rather than aerobic incubation.

## Principle
Tryptone, yeast extract and L-cystine provide sources of nitrogen, carbon and other growth factors while dextrose is the carbohydrate source. Sodium chloride provides essential ions and maintains the osmotic balance. Sodium thioglycollate is a reducing agent, which prevents the accumulation of peroxides that is lethal to bacterial growth and neutralizes the antibacterial effect of mercurial preservatives. L-cystine is also a reducing agent, since it contains sulphydryl groups that inactivate heavy metal compounds, which exert a bacteriostatic effect in the materials under examination, and also maintains a low redox potential, thereby maintaining anaerobiosis. Absence of agar makes it a suitable medium for sterility testing of viscous materials and devices having tubes with small lumina.

## Formula*

| **Ingredients** | **g/L** |
|---|---|
| Tryptone | 15.0 |
| Sodium Thioglycollate | 0.5 |
| Dextrose | 5.5 |
| Sodium Chloride | 2.5 |
| Yeast Extract | 5.0 |
| L-Cystine | 0.5 |
| Final pH (at 25°C) | 7.1 ± 0.2 |

*Adjusted to suit performance parameters

## Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

## Type of specimen
Clinical - wound swabs, skin swabs or scrapings, tooth tartar etc.
Pharmaceutical - Sterility testing of viscous products.

## Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

## Directions

1. Suspend 29.00 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Heat with the frequent agitation and boil for one minute to dissolve the powder completely.
4. Dispense as desired into containers.
5. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
6. Tighten lids of the containers immediately (while still warm) to reduce oxidation.
7. Cool to 25°C and store in a cool dark place preferably below 25°C.

## Quality Control

**Dehydrated Appearance**: Yellow coloured, homogeneous and free flowing powder.
**Prepared Appearance**: Yellow coloured, clear solution without any precipitate.
**Growth Promotion Test**: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C - 35°C for ≤ 3 days.
**Growth Promoting Properties**: The test results observed are within the specified temperature and shortest period of time, inoculating ≤ 100 cfu (at 30°C - 35°C for ≤ 3 days).

| **Organism (ATCC)** | **Growth** |
|---|---|
| *Bacillus spizizenii* (6633) | Good |
| *Bacteroides fragilis* (25285) | Good |
| *Bacteroides vulgatus* (8482) | Good |
| *Clostridium sporogenes* (11437) | Good |
| *Pseudomonas aeruginosa* (9027) | Good |
| *Staphylococcus aureus* subsp. *aureus* (6538) | Good |
| *Streptococcus pyogenes* Strain Bruno (19615) | Good |
| *Candida albicans* 3147 (10231) | Good |
| *Aspergillus brasiliensis* WLRI 034(120) (16404) | Good |
| *Kocuria rhizophila* Strain PCI 1001 (9341) | Good |

**Note:** Inoculum cfu for good growth is 10-100.

## Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

## Interpretation of Result

1. Upon incubation, growth is indicated by the presence of turbidity compared to an uninoculated control.
2. Subcultures to appropriate solid media should be made to obtain pure cultures of isolates, which can then be further tested and identified.

**Note**: inspect the tubes for clarity before inoculation. Ensure that the tubes are labeled correctly with the respective sample.

## Precautions / Limitations

1. Some dextrose fermenting organisms, which are able to reduce the pH of the medium to a critical level, may not survive in this medium. Early subculture is required to isolate these organisms.
2. In test samples, the proper surface to volume ratio of the medium must be maintained to avoid oxidation of the medium, which is unsuitable for microaerophilic and anaerobic growth.
3. Anaerobes can be overgrown by more rapidly growing facultative organisms. Gram stain and examine the broth if plating medium reveals no growth.
4. The growth of some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.
5. Do not rely on broth cultures exclusively for isolation of anaerobes.
6. Do not reheat the medium more than once as it may give rise to toxicity.

## Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## Reference


1. N.I.H. Memorandum, 1955: Culture Media for Sterility Tests, 4th Revision.
2. Nungester, Hood and Warren, 1943, Proc. Soc. Exp. Biol. Med., 52: 287.
3. Portwood, 1944, J. Bacteriol., 48: 255.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.


## Product Presentation:

| Cat No. | Product description | Pack Size |
|---|---|---|
| 201010080100 | Dehydrated Culture Media | 100 g |
| 201010080500 | Dehydrated Culture Media | 500 g |
| 203010460100 | Bottle Media (Screw Cap) | 100 mL |
| 203010470100 | Bottle Media (Canister) | 100 mL |