



**BBL™ Thioglycollate Medium, Fluid
without Dextrose or Eh Indicator
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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Thioglycollate Medium, Fluid without Dextrose or Eh Indicator is useful in the performance of fermentation studies of anaerobic organisms when supplemented with appropriate carbohydrates.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Loosen the caps and place the tubes in boiling water for 1–2 min to reduce the medium. Tighten the caps immediately after removing from the heat and allow the medium to cool to room temperature prior to use.
 - b. Using sterile 1.0 mL pipettes, inoculate tubes with 1.0 mL of dilutions of 18- to 24-h broth cultures. Use **Trypticase™** Soy Broth for the *Staphylococcus* and *Bacillus* cultures and Thioglycollate without Indicator-135C for the *Clostridium* strain. The dilution used should contain 1000 or less CFU/mL.
 - c. Incubate tubes with tightened caps (loosened caps for the *Staphylococcus* and *Bacillus* strains) at 35 ± 2°C in an aerobic atmosphere.
2. Examine tubes for up to 7 days for growth.
3. Expected Results

Organisms	ATCC™	Recovery
* <i>Clostridium sporogenes</i>	11437	Growth
<i>Bacillus subtilis</i>	6633	Growth
* <i>Staphylococcus aureus</i>	25923	Growth

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Incubate uninoculated representative tubes aerobically at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Thioglycollate Medium, Fluid without Dextrose or Eh Indicator is used for fermentation studies, especially with anaerobic organisms.

V SUMMARY AND EXPLANATION

This modification of basic Thioglycollate Medium,^{1,2} due to the absence of dextrose and the use of casein peptone free of fermentable carbohydrates, is well suited for conducting fermentation studies on anaerobes. Filter-sterilized carbohydrate solutions can be added aseptically to sterile broth prior to inoculation or **BBL™ Taxo™** Carbohydrate Discs can be added to the sterile medium.

In this formulation, the Eh indicator, methylene blue, is also omitted to avoid any toxicity problems and to facilitate early recognition of growth.

VI PRINCIPLES OF THE PROCEDURE

Sodium thioglycollate creates an atmosphere of low oxygen tension in this medium, thereby enabling obligate anaerobes, as well as other organisms, to grow. The casein peptone and cystine supply essential nitrogenous and carbon compounds. Sodium chloride provides essential ions. The small amount of agar aids in the prevention of convection currents in the medium, contributing to the maintenance of anaerobiosis.

VII REAGENTS

Thioglycollate Medium, Fluid without Dextrose or Eh Indicator

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	20.0 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 g
L-Cystine	0.5 g
Agar	0.75 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

IX PROCEDURE

Material Provided: Thioglycollate Medium, Fluid without Dextrose or Eh Indicator

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Liquid media for anaerobic incubation should be reduced prior to inoculation by placing the tubes, with caps loosened, under anaerobic conditions for 18–24 h prior to use. An efficient and easy way to obtain suitable anaerobic conditions is through the use of the **BD GasPak™** EZ anaerobic system. Alternatively, liquid media may be reduced immediately prior to use by boiling, with caps loosened, and cooling, with tightened caps, to room temperature before inoculation.

Inoculate tubes of the medium containing various carbohydrates (0.5–1.0%) with 24- to 48-h pure cultures. Incubate tubes with loosened caps (aerobes) for 24–48 h at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere or with tightened caps (anaerobes).

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed, bottled and **Mycoflask™** brand media. The tip of the electrode should be placed below the surface of broth media.

X RESULTS

After a sufficient incubation period, examine the tubes for the presence of growth as evidenced by turbidity in the medium. Comparative acid and pH determinations may be made at intervals during incubation in order to determine the ability of the organism to ferment the carbohydrate which has been added.

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.³⁻⁸

XII AVAILABILITY

Cat. No.	Description
221398	BBL™ Thioglycollate Medium, Fluid without Dextrose or Eh Indicator, Ctn. of 100 size K tubes

XIII REFERENCES

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