# PRODUCT SPECIFICATION SHEET

## Brewer Thioglycollate Medium, Modified (Thioglycollate Medium,Linden) (DM265)

**Intended Use**

Brewer Thioglycollate Medium, Modified (Thioglycollate Medium,Linden) (DM265) is recommended for testing sterility of biological products and for isolation of a wide variety of aerobic and anaerobic organisms..

**Product Summary and Explanation**

Quastel and Stephenson[1] noticed that the presence of a small amount of a compound containing an –SH group (cysteine, thioglycollic acid, glutathione) allowed "aerobic" growth of *Clostridium sporogenes* in tryptic digest broth.
Falk, Bucca and Simmons[2] brought out the advantages of using small quantities of agar (0.06-0.25%) in detecting contaminants during sterility testing of biologicals. Brewer[3] demonstrated the value of combining a small amount of agar and a reducing substance. Brewer's experiments revealed that anaerobes grew equally well, in a liquid medium containing 0.05% agar, irrespective of presence or absence of sodium thioglycollate. Marshall, Gunnish and Luxen[4] reported satisfactory cultivation of anaerobes in Brewer's Thioglycollate Medium in the presence of a mercurial preservative. Neutralization of the bacteriostatic effect of mercurial compounds by sodium thioglycollate was confirmed by Nungester, Hood and Warren[5] and Portwood.[6] Vera[7] introduced incorporation of casein peptone. Malin and Finn[8] reported the commonly used medium containing thioglycollate is inhibitory to some organisms in the presence of a carbohydrate.
Fluid Thioglycollate Medium is recommended in the FDA *Bacteriological Analytical Manual* (BAM)[9] and the *Official Methods of Analysis of AOAC International*[10] for the examination of food, and for determining the phenol coefficient and sporicidal effects of disinfectants. Fluid Thioglycollate Medium is also specified for sterility checks on banked blood.[11] It is one of the media recommended in the *USP* for use in sterility testing of articles purporting to be sterile; these formulations meet the requirements of the *USP* growth promotion test.[12]
In 1941, the National Institutes of Health specified the use of two thioglycollate media in sterility testing, the Brewer Formula and the Linden Formula.[13] The Linden Formula was later referred to as Modified Brewer Thioglycollate Medium in which meat infusion was replaced by plant (soy) peptones.[14]

**Principles of the Procedure**

Brewer Thioglycollate Medium, Modified contains highly nutritious casein enzymic hydrolysate and papaic digest of soyabean meal which supports luxuriant growth of fastidious bacteria. Dextrose is the fermentable carbohydrate and energy source. Sodium thioglycollate aids in creating anaerobic condition and also neutralizes toxicity of mercurial compounds if present in the inoculum of the test sample. Sodium chloride maintains the osmotic equilibrium of the medium. Dipotassium phosphate serves as a buffering agent. Very small amount of agar present maintains anaerobic conditions at the bottom of the broth. Methylene blue indicates oxygen content of the medium by exhibiting bluish-green colour to the medium in presence of oxygen. In an uninoculated medium bluish green colour at the top indicates presence of oxygen in that part. The medium contains more thioglycollate and is recommended for sterility testing procedures. Organisms that ferment dextrose and lower the pH to critical levels may not survive in this medium after growth has taken place.

**Formula / Liter**

| Ingredients | Gms / Liter |
|---|---|
| Casein enzymic hydrolysate | 17.50 |
| Papaic digest of soyabean meal | 2.50 |
| Dextrose | 10.00 |
| Sodium chloride | 5.00 |

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|---|---|
| Dipotassium phosphate | 2.00 |
| Sodium thioglycollate | 1.00 |
| Methylene blue | 0.002 |
| Agar | 0.50 |
| Final pH: 7.2 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

**Precautions**

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or in free flowing steam until the pink colour disappears.
4. Do not reheat the media more than once; continued reheating gives rise to toxicity.

**Directions**

1. Suspend 29.75 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 25°C and store in a cool dark place preferably below 25°C.

**Quality Control Specifications**

| | |
|---|---|
| **Dehydrated Appearance** | Cream to yellow homogeneous free flowing powder |
| **Prepared Medium** | Yellow coloured clear to slightly opalescent fluid with upper 10% or less medium bluish green on standing |
| **Reaction of 3.85% Solution** | pH : 7.2 ± 0.2 at 25°C |
| **Gel Strength** | Not Applicable |

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours (Clostridium and Bacteroides species incubated anaerobically).

| Sr. No. | Organisms | Results to be achieved | |
|---|---|---|---|
| | | Inoculum (CFU) | Growth |
| 1. | *Bacteroides melaninogenicus ATCC 25848* | 50 - 100 | good-luxuriant |
| 2. | *Clostridium sporogenes ATCC 11437* | 50 - 100 | good-luxuriant |
| 3. | *Streptococcus mitis ATCC 9895* | 50 - 100 | good-luxuriant |
| 4. | *Streptococcus pyogenes ATCC 19615* | 50 - 100 | good-luxuriant |
| 5. | *Bacteroides fragilis ATCC 25285* | 50 - 100 | good-luxuriant |
| 6. | *Staphylococcus aureus ATCC 25923* | 50 - 100 | good-luxuriant |

The organisms listed are the minimum that should be used for quality control testing.

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**Test Procedure**
Refer to appropriate references for standard test procedures.

**Results**
1. After incubation, growth is evidenced by the presence of turbidity compared to an uninoculated control.
2. Strict aerobes tend to grow in a thin layer at the surface of the broth; obligate anaerobes will grow only in that portion of the broth below the upper oxidized layer.

**Storage**
Store the sealed bottle containing the dehydrated medium at 10- 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

**Expiration**
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

**Limitations of the Procedure**
1. Anaerobes can be overgrown by more rapidly growing facultative organisms.
2. If plating medium reveals no growth examine and Gram stain broth.
3. Never rely on broth cultures exclusively for isolation of anaerobes. Some anaerobes may be inhibited by metabolic products or acids produced from more rapidly growing facultative anaerobes.
4. Consult appropriate texts for detailed information and recommended procedures.

**Packaging**
**Product Name : Brewer Thioglycollate Medium, Modified (Thioglycollate Medium,Linden)**
**Product Code : DM265**
**Available Pack sizes : 100gm/ 500gm**

**References**

1. Quastel and Stephenson. 1926. J. Biochem. *20*:1125.
2. Falk, Bucca and Simmons. 1939. J. Bacteriol. *37*:121.
3. Brewer. 1940. JAMA *115*:598.
4. Marshall, Ginnish and Luxen. 1940. Proc. Soc. Exp. Biol. Med. *43*:672.
5. Nungester, Hood and Warren. 1943. Proc. Soc. Exp. Biol. Med. 52:287.
6. Portwood. 1944. J. Bacteriol. *48*:255.
7. Vera. 1944. J. Bacteriol. *47*:59.
8. Malin and Finn. 1957. J. Bacteriol. *62*:349.
9. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
10. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
11. Federal Register. 1992. Fed. Regist. *21*:640.2.17.
12. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
13. Linden. 1941. Fluid thioglycollate medium for the sterility test. National Institutes of Health, Bethesda, Md.
14. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

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**Further Information**

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