PRODUCT SPECIFICATION SHEET

Fluid Thioglycollate Medium (DM263B)

Intended Use

Fluid Thioglycollate Medium (DM263B) is recommended for sterility testing of biologicals and for cultivation of aerobes, anaerobes and microaerophiles in compliance with BP.

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.

The British Pharmacopoeia[9] USP,[10] EP[11] and AOAC[12] have recommended the media for sterility testing of antibiotics, biologicals and foods and for determining the phenol coefficient and sporicidal effect of disinfectants. However, it is intended for the examination of clear liquid or water-soluble materials.

Principles of the Procedure

Fluid Thioglycollate medium contains pancreatic digest of casein, yeast extract, L-cystine which provides the growth factors necessary for bacterial multiplication. Glucose monohydrate is the carbon and energy source. L-cystine and sodium thioglycollate allows *Clostridium* to grow in this medium even under aerobic conditions. Small amount of agar added in the medium favors the growth of aerobes as well as anaerobes in the medium, even if sodium thioglycollate is deleted from the medium and also helps in maintaining low redox potential for stabilizing the medium. Sodium thioglycollate act as a reducing agent and neutralizes the toxic effects of mercurial preservatives and peroxides formed in the medium, thereby promoting anaerobiosis, and making the medium suitable to test materials containing heavy metals. Resazurin is an indicator dye when oxygen content increases slightly, it is indicated by a colour change of redox indicator, resazurin to red.[4,5,6,13]

Formula / Liter

Ingredients	Gms / Liter
Pancreatic digest of casein	15.00
Yeast extract	5.00
Glucose monohydrate	5.50
Sodium chloride	5.00
L-Cystine	0.50
Sodium thioglycollate	0.50
Resazurin sodium	0.001
Agar	0.75
Final pH: 7.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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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 29.25 grams of the medium in one liter of R Water/purified/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	Light straw coloured clear to slightly opalescent solution with upper 10% or less medium pink on standing
Reaction of 2.92% Solution	pH : 7.1 ± 0.2 at 25°C
Gel Strength	Not Applicable

Growth Promotion Test

As per British Pharmacopoeia

Growth promoting properties

Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at 30-35°C for <=3 days.

Stability test

Light yellow coloured clear solution without any precipitation sedimentation at room temperature for 7 days.

Expected Cultural Response: Cultural characteristics observed after an incubation at 30-35°C for not more than 3 days.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
	When incubated anaerobically		
1.	*Clostridium sporogenes ATCC 19404*	50 – 100	good-luxuriant
2.	*Clostridium sporogenes ATCC 11437*	50 – 100	good-luxuriant
3.	*Clostridium sporogenes NBRC 14293*	50 – 100	good-luxuriant
4.	*Clostridium perfringens ATCC 13124*	50 – 100	good-luxuriant
5.	*Bacteoides fragilis ATCC 23745*	50 – 100	good-luxuriant
6.	*Bacteroides vulgatus ATCC 8482*	50 – 100	good-luxuriant
	When incubated aerobically		
7.	*Staphylococcus aureus ATCC25923*	50 – 100	good-luxuriant

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8.	*Staphylococcus aureus ATCC 6538*	50 – 100	good-luxuriant
9.	*Pseudomonas aeruginosa ATCC 27853*	50 – 100	good-luxuriant
10.	*Pseudomonas aeruginosa ATCC 9027*	50 – 100	good-luxuriant
11.	*Micrococcus luteus ATCC 9341*	50 – 100	good-luxuriant
12.	*Streptococcus pneumonia ATCC 6305*	50 – 100	good-luxuriant
13.	*Escherichia coli ATCC 25922*	50 – 100	good-luxuriant
14.	*Escherichia coli ATCC 8739*	50 – 100	good-luxuriant
15.	*Escherichia coli NCTC 9002*	50 – 100	good-luxuriant
16.	*Salmonella Typhimurium ATCC 14028*	50 – 100	good-luxuriant
17.	*Salmonella Abony NCTC 6017*	50 – 100	good-luxuriant
18.	*Bacillus subtilis ATCC 6633*	50 – 100	good-luxuriant

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

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 at10- 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 : Fluid Thioglycollate medium
Product Code : DM263B
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.
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6. Portwood. 1944. J. Bacteriol. *48*:255.
7. Vera. 1944. J. Bacteriol. *47*:59.
8. Malin and Finn. 1957. J. Bacteriol. *62*:349.
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10. U.S. Pharmacopoeia, 2011, United States Pharmacopoeia Convention, Inc., Rockville, MD.
11. European Pharmacopoeia, 2011, European Department, for the Quality of Medicines.
12. Williams. (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th ed., AOAC, Washington, D.C.
13. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of ,,Medical Bacteria, Vol.1, Williams and Wilkins, Baltimore.

Further Information

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