



PRODUCT SPECIFICATION SHEET

Fluid Thioglycollate Medium w/ Beef Extract (DM500)

Intended Use

Fluid Thioglycollate Medium w/ Beef Extract (DM500) is recommended for cultivation of anaerobic, microaerophilic and aerobic microorganisms and for sterility testing

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⁾ Fluid Thioglycollate Medium with Beef Extract is recommended for the detection of viable bacteria in live vaccines, as recommended by the Animal and Plant Health Inspection Services, USDA.⁽¹³⁾

Principles of the Procedure

Fluid Thioglycollate Medium w/ Beef Extract contains casein enzymic hydrolysate, yeast extract, beef extract, L-cystine which provides the growth factors necessary for bacterial multiplication. Dextrose is the carbon and energy source. 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. 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. It also helps in maintaining low redox potential for stabilizing the medium. Resazurin is an indicator dye when oxygen content increases slightly, it is indicated by a colour change of redox indicator, resazurin to red.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	15.00
Yeast extract	5.00
Beef extract	5.00
Dextrose	5.50
L-Cystine	0.50
Sodium chloride	2.50
Sodium thioglycollate	0.50
Resazurin sodium	0.001





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Agar	0.75
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 34.75 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	Light straw coloured, clear to slightly opalescent solution with upper 10% or less medium pink on standing
Reaction of 3.47% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 25-30°C for 40-72 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Bacillus subtilis</i> ATCC 6633	50-100	good-luxuriant
2.	<i>Candida albicans</i> ATCC 10231	50-100	good-luxuriant
3.	<i>Clostridium sporogenes</i> ATCC 11437	50-100	good-luxuriant
4.	<i>Micrococcus luteus</i> ATCC 10240	50-100	good-luxuriant
5.	<i>Bacteroides vulgatus</i> ATCC 8482	50-100	fair-good
6.	<i>Neisseria meningitidis</i> ATCC 13090	50-100	good-luxuriant
7.	<i>Streptococcus pyogenes</i> ATCC 19615	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.





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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 : Fluid Thioglycollate Medium w/ Beef Extract

Product Code : DM500

Available Pack sizes : 500gm

References

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8. Malin and Finn. 1957. J. Bacteriol. 62:349.
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11. Federal Register. 1992. Fed. Regist. 27:640.2.17.
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. Federal Register, 1992, Fed. Regist., 27:113.26.

Further Information

For further information please contact your local MICROMASTER Representative.



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