IFU

Instructions for Use

FLUID THIOGLYCOLLATE, ENRICHED

Cat. no. K73	Fluid Thioglycollate, Enriched, 16x125mm Tube, 10ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Fluid Thioglycollate, Enriched contains Hemin and Vitamin K and is recommended for use as a highly nutritious general purpose growth medium for the cultivation of aerobic, microaerophilic and anaerobic microorganisms from sterile materials.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Fluid Thioglycollate was first described in 1940 by Brewer and the medium is useful for testing normally sterile materials.[4,5,8,9] Agar in the formula promotes the growth of small inocula and anaerobes by impeding the diffusion of oxygen within the medium; it also restricts the dispersion of CO_2 and reducing substances from the microenvironment surrounding the inoculum. Sodium thioglycollate is a reducing agent used to maintain low oxygen tension by removing molecular oxygen: peroxides, which may be lethal to many anaerobic organisms, do not form under this condition. Cystine and casein supply carbon and nitrogenous compounds, dextrose is added as an energy source, and sodium chloride maintains osmotic equilibrium.

Hardy Diagnostics Fluid Thioglycollate, Enriched medium contains resazurin as an oxidation-reduction indicator that turns pink when increased oxidation has occurred. Yeast extract, or papaic digest of soybean meal, is added as a growth enhancer. Fluid Thioglycollate, Enriched includes hemin to supply X-factor for the growth of many fastidious microorganisms and vitamin K to promote the growth of some gram-positive spore-formers and *Bacteroides* species.[2,3,6,7]

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Dextrose	5.5gm
Yeast Extract	5.0gm
Sodium Chloride	2.5gm
L-Cystine	0.5gm
Sodium Thioglycollate	0.5gm
Hemin	5.0mg

Resazurin Indicator	1.0mg
Vitamin K	0.1mg
Agar	0.75gm

Final pH 7.1 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[2,3,6,7] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated to an appropriate transport media and refrigerated until inoculation.

Method of Use: Consult listed references for the appropriate cultivation techniques using this medium.[2,3,6,7] It is recommended that liquid media for anaerobic incubation be reduced prior to inoculation by placing tubes (with loosened caps) under anaerobic conditions for 18-24 hours. Alternately, the boiling method described below may be used. Fluid Thioglycollate, Enriched medium should be incubated at 35-37 degrees C. for up to one week and checked daily for signs of growth. Growth or turbidity should be confirmed by Gram stain and subculture onto an appropriate growth medium. Broth cultures should be held for one week before being discarded as negative.

Note: Fluid Thioglycollate, Enriched contains a resazurin indicator which will cause the upper layer of the broth to

become pink when exposed to oxygen. Containers that have been agitated recently (as during shipping) will turn pink throughout. This can be reversed by allowing the container to stand still for a few hours or by placing tubes in a boiling waterbath for 10 minutes with loosened caps. The caps should be tightened firmly before the media cools. Also note that a whitish precipitate may form in this medium due to the agar content. This does not affect the performance of this medium and may dissipate when the tubes are heated.

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth and other indicator tests used to identify growth of organisms in this medium.[2,3,6,7]

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

It is recommended that the proper surface to volume ratio of Fluid Thioglycollate, Enriched medium be maintained to avoid oxidation, making it unsuitable for microaerophilic and anaerobic growth.[2]

A slight turbidity or haziness may be present due to the small amount of agar in the medium. When the medium has been boiled it appears clear.

Do not boil the medium more than once, as frequent boiling may lead to the production of toxic by-products.[7] If it is suspected that the medium has more than 30% oxidation **after** boiling, it should be discarded.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Clostridium sporogenes ATCC® 19404***	J	24-48hr	35°C	Aerobic**	Growth
Staphylococcus aureus ATCC® 6538***	J	24hr	35°C	Aerobic**	Growth
Pseudomonas paraeruginosa ATCC® 9027	J	24hr	35°C	Aerobic**	Growth
Bacteroides fragilis ATCC® 25285	J	24-48hr	35°C	Aerobic**	Growth

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Tubes are incubated in an aerobic incubator with the caps screwed down tightly to create an atmosphere of low oxygen tension within the tube.

*** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Fluid Thioglycollate, Enriched should appear clear, and light amber in color. Tubes with oxygen present will have a pink layer at the medium-air interface. If the medium appears pink in color, follow the instructions given in the above "Procedure" section to reduce the medium prior to use.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen, et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Brewer, J.H. 1940. *J. Amer. Med. Assoc.;* 115:598.

5. Federal Security Agency, Food and Drug Administration, Compilation of Regulations for Test and Methods of Assay and Certification of Antibiotic Drugs.

6. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

7. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

8. National Formulary, 9th ed. p.768, 1950.

9. National Institutes of Health Circular: Culture Media for the Sterility Test, 2nd rev. Feb. 5, 1946.

10. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207D [D]