IFU

Instructions for Use

FLUID THIOGLYCOLLATE MEDIA, USP

Cat. no. K84	Fluid Thioglycollate, USP/EP/JP, 20x125mm Tube, 15ml	20 tubes/box
Cat. no. K121	Fluid Thioglycollate, USP, 20x125mm Tube, 10ml	20 tubes/box
Cat. no. K212BX	Fluid Thioglycollate, USP, 16x125mm Tube, 10ml	80 tubes **ReadyRack
Cat. no. K282	Fluid Thioglycollate, USP, 16x125mm Tube with Hungate Cap, 10ml	20 tubes/box
Cat. no. U41	Fluid Thioglycollate, USP, 8oz. Wide Mouth Glass Jar, 100ml	12 jars/box
Cat. no. U43*	Fluid Thioglycollate without Indicator, USP, 4oz. Glass Bottle, 100ml	20 bottles/box
Cat. no. U66	Fluid Thioglycollate, USP, 20ml Serum Vial, 15ml	50 vials/box
Cat. no. U68	Fluid Thioglycollate, USP, 20ml Serum Vial, 20ml	50 vials/box
Cat. no. U84	Fluid Thioglycollate, USP, 100ml Serum Vial, 100ml	20 vials/box
Cat. no. U121	Fluid Thioglycollate, USP, 4oz. Glass Bottle, 100ml	20 bottles/box
Cat. no. U207	Fluid Thioglycollate, USP, 200ml Serum Vial with Septum Cap, 150ml	10 vials/box
Cat. no. U273	Fluid Thioglycollate, USP, 500ml Glass Bottle with Septum Cap, 500ml	10 bottles/box
Cat. no. U427	Fluid Thioglycollate, USP, 500ml Glass Bottle with Septum Cap, 300ml	10 bottles/box
Cat. no. U434	Fluid Thioglycollate, USP, 8oz Boston Round Glass Bottle, 200ml	12 bottles/box
Cat. no. U479	Fluid Thioglycollate, USP, 2oz. Glass Bottle, 40ml	24 bottles/box

* Product does not contain resazurin indicator.

** ReadyRack is a plastic rack designed for cleanroom use and can be rinsed with alcohol.

INTENDED USE

Hardy Diagnostics Fluid Thioglycollate Media, USP is recommended for the cultivation of aerobic, microaerophilic, and anaerobic microorganisms in normally sterile materials. Fluid Thioglycollate Medium conforms to the formula stated in the United States Pharmacopeia (USP).[4]

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

SUMMARY

The Fluid Thioglycollate Medium formulation was first described in 1940 by Brewer.[1] This medium demonstrated that combining a small amount of agar and a reducing substance initiated the growth of anaerobic bacteria. The Fluid Thioglycollate formulation is the standard medium recommended by the Food and Drug Administration, National

Institute of Health, the National Formulary, and the U.S. Pharmacopeia for sterility testing of clear fluid biologics and other sterile products.[2,4,5]

The addition of a small amount of agar in the media aids in the initiation and growth of small inocula and anaerobes by impeding the diffusion of oxygen into the medium. It also retards the dispersion of CO_2 and the reducing substance from the microenvironment surrounding the inoculum. Sodium thioglycollate is a reducing agent which maintains a low oxygen tension by removing molecular oxygen from the environment. Peroxides, which may be lethal to many anaerobic organisms, are not formed under this condition. Cystine and casein supply carbon and nitrogenous compounds, dextrose is added as another energy source, and sodium chloride maintains osmotic equilibrium.

Certain additives can be incorporated into the Fluid Thioglycollate Medium as desired. Resazurin is an oxidation-reduction indicator that turns pink when increased oxidation has occurred. Yeast extract, papaic digest of soybean meal or beef extract are added as growth enhancers. Lecithin and Tween® 80 are added to neutralize germicidal or disinfectant residues; neutralization of these residues reduces their inhibitory effect. Quaternary ammonium compounds are neutralized by lecithin while phenolic disinfectants and hexachlorophene are neutralized by Tween® 80. Together, lecithin and Tween® 80 neutralize ethanol.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Dextrose	5.5gm
Yeast Extract	5.0gm
Sodium Chloride	2.5gm
Sodium Thioglycollate	0.5gm
L-Cystine	0.5gm
Agar	0.75gm

If added:

Resazurin indicator**	1.0mg

Final pH 7.1 +/- 0.2 at 25ºC.

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

** For those products that do not contain resazurin indicator, final pH is 7.2 +/- 0.2 at 25ºC.

Formulated in accordance with USP <71>.[8]

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-25ºC away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat 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

In some tubes, the media may become oxidized within the tube during shipment. Resazurin, an oxidation-reduction indicator causes the entire medium to turn pink when oxidation has occurred. These tubes may be restored to their proper condition by bringing the media up to 100ºC in a boiling waterbath. Loosen screw caps slightly before heating, and tighten during cooling to room temperature. The boiling serves to reduce media intended for the culture of anaerobic organisms.

Method of Use: Consult the U.S. Pharmacopeia for sterility testing procedures.[4]

Note: Fluid Thioglycollate contains a resazurin indicator which will cause the upper 1/3 layer of this broth to be pink, due to exposure 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 putting the containers in a boiling waterbath for 10 minutes with loosened caps. The caps are then tightened firmly before the media cools. Also note that it is common for a whitish precipitate to form in this medium due to the agar content. This does not effect the performance of this medium.

INTERPRETATION OF RESULTS

Consult U.S. Pharmacopeia for interpretation guidelines for testing.[4]

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.

In test samples, the proper surface to volume ratio of the Fluid Thioglycollate Medium must be maintained to avoid oxidation of the medium, making it unsuitable for microaerophilic and anaerobic growth.[4]

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

Do not boil the media more than once, as frequent boiling may lead to toxic products forming in the medium.[3] 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, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as indicators, 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	
Staphylococcus aureus ATCC® 6538	J	24-72hrs	30-35°C	Aerobic	Growth
Pseudomonas aeruginosa ATCC® 9027	J	24-72hrs	30-35°C	Aerobic	Growth
Clostridium sporogenes ATCC® 19404	J	24-72hrs	30-35°C	Aerobic**	Growth

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

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

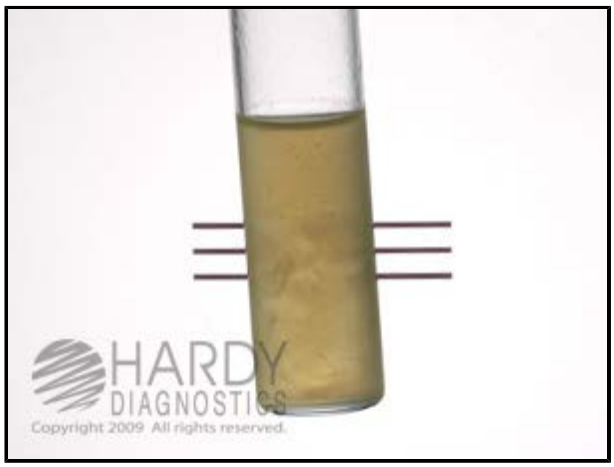
*** Tested in accordance with USP <61> and <62>.[6,7]

USER QUALITY CONTROL

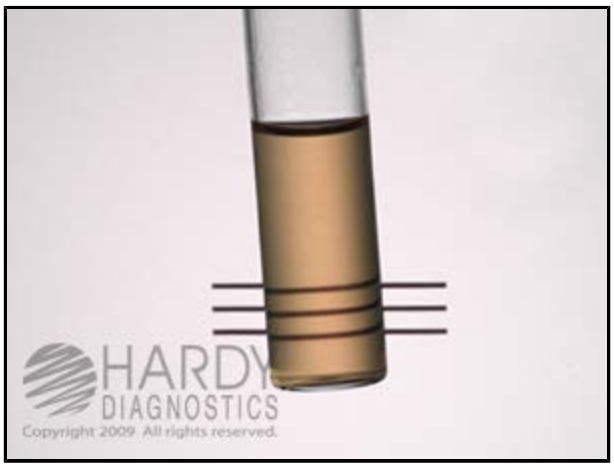
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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 Medium should appear translucent, and light amber in color. Containers with oxygen present will have a pink layer at the medium-air interface. If the media appears pink in color, follow the instructions given in the above "Procedure" section to restore and reduce the media.



Clostridium sporogenes (ATCC® 19404) growing in Fluid Thioglycollate Media, USP (Cat. no. K121). Incubated aerobically with tightened cap for 24 hours at 35ºC.



Uninoculated tube of Fluid Thioglycollate Media, USP (Cat. no. K121).

REFERENCES

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

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

3. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.

4. The Official Compendia of Standards. *USP-NF*. United States Pharmacopeial Convention, Rockville, MD.

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

6. The Official Compendia of Standards. USP General Chapter <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests. *USP-NF*. United States Pharmacopeial Convention Inc., Rockville, MD.

7. The Official Compendia of Standards. USP General Chapter <62> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms. *USP-NF*. United States Pharmacopeial Convention Inc., Rockville, MD.

8. The Official Compendia of Standards. USP General Chapter <71> Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms. *USP-NF*. United States Pharmacopeial Convention Inc., Rockville, MD.

IFU-10433[C]

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207H [D]