IFU

Instructions for Use

THIOGLYCOLLATE MEDIA

Cat. no. K21	Thioglycollate with Indicator, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. K22	Thioglycollate with H & K, 16x100mm Tube, 5ml	20 tubes/box
Cat. no. K24	Thioglycollate with H & K, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. K23	Thioglycollate with Supplements, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. K28	Thioglycollate with Supplements, 20x125mm Tube, 15ml	20 tubes/box
Cat. no. K29	Thioglycollate without Indicator, 16x125mm Tube, 10ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Thioglycollate Media is recommended for the cultivation of aerobic, microaerophilic, and anaerobic microorganisms.

SUMMARY

The addition of a small amount of agar in Thioglycollate Medium 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 are incorporated into the Thioglycollate Medium as desired. Resazurin is an oxidation-reduction indicator that turns pink when increased oxidation has occurred. Yeast extract or papaic digest of soybean meal are added as growth enhancers. Hemin is incorporated to supply X-factor for the stimulated growth of many fastidious organisms, and vitamin K because it is a growth requirement for some gram-positive spore-formers and *Bacteroides* species. The calcium carbonate chip is added to act as a buffer for the medium and prevents a buildup of toxic acid.

FORMULA

Ingredients per liter of deionized water:*

Thioglycollate with Indicator:	
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
Resazurin	1.0mg
Agar	0.75gm

Final pH 7.1 +/- 0.3 at 25ºC.

Thioglycollate without Indicator, Thioglycollate with H & K, Thioglycollate with Supplements:	
Pancreatic Digest of Casein	15.0gm
Dextrose	5.0gm
Yeast Extract	5.0gm
Sodium Chloride	2.5gm
Sodium Thioglycollate	0.5gm
L-Cystine	0.25gm
Agar	0.75gm

In addition;

Thioglycollate with H & K contains:	
Hemin	5.0mg/L
Vitamin K	1.0mg/L
Thioglycollate with Supplements contains:	
Hemin	5.0mg/L
Vitamin K	1.0mg/L
Calcium Carbonate Chip	1/tube

Final pH 7.2 +/- 0.3 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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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.[1-3,6] 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 onto an appropriate transport media and refrigerated until inoculation.

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 listed references for the appropriate cultivation techniques using this medium.[1-3,6] It is recommended that liquid media for anaerobic incubation should be reduced prior to inoculation by placing tubes (with loosened caps) under anaerobic conditions for 18-24 hours. Alternatively, the boiling method described above may be used. Thioglycollate Medium should be incubated at 35-37ºC., checking daily, as needed. Growth or turbidity should be confirmed by gram stain and subculture onto an appropriate growth medium.

Note: Thioglycollate with Indicator Media contains a resazurin indicator which will cause the upper layer of this broth to be pink, due to the 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 affect the performance of this medium.

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth and other identification tests to identify growth of organism in this medium.[1-3,6]

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 Thioglycollate Medium must be maintained to avoid oxidation of the medium, making it unsuitable for microaerophilic and anaerobic growth.

A slight turbidity or 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 media more than once, as frequent boiling may lead to toxic products forming in the medium.[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, other culture media, swabs, applicator sticks, 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	
Thioglycollate with Indicator, Thioglycollate without Indicator:					
Clostridium novyi A ATCC® 7659***	A	24-48hr	35°C	Aerobic**	Growth
Staphylococcus aureus ATCC® 25923	A	24hr	35°C	Aerobic**	Growth
Candida albicans ATCC® 10231	A	24hr	35°C	Aerobic**	Growth
Bacillus subtilis ATCC® 6633	A	24hr	35°C	Aerobic**	Growth
Thioglycollate with H & K, Thioglycollate with Supplements:					
Clostridium perfringens ATCC® 13124***	A	48hr	35°C	Aerobic**	Growth
Bacteroides levii ATCC® 29147	A	48hr	35°C	Aerobic**	Growth
Bacteroides vulgatus ATCC® 8482	A	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.

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 2207F [D]