anaeroGRO™
Pre-Reduced Anaerobic Culture Media

THIOGLYCOLLATE WITH HEMIN AND VITAMIN K (H AND K) WITHOUT INDICATOR

Cat. no. AG22H	Thioglycollate with H and K, 16x125mm Tube with Hungate Septum Cap, 9ml	20 tubes/box
Cat. no. AG23H	Thioglycollate with H and K, 16x125mm Tube with Hungate Septum Cap, 7ml	20 tubes/box

INTENDED USE

Hardy Diagnostics AnaeroGRO™ Thioglycollate with Hemin and Vitamin K (H and K) is recommended for the cultivation of microaerophilic, facultative and obligate anaerobic microorganisms.

SUMMARY

Thioglycollate media is recommended for the use in cultivation and isolation of fastidious or slow glowing obligately anaerobic microorganisms present in clinical specimens. The cultivation of microorganisms are enhanced by the use of enriched broth media in addition to the selective and differential plate media normally used for primary isolation.

Thioglycollate medium contains small amounts of agar which aids in the initiation of growth of anaerobes by impeding the diffusion of oxygen into the medium from the air/liquid interface. 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. Yeast extract is added as a growth enhancer. Hemin is incorporated to supply the X-factor for 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.

Hardy Diagnostics Thioglycollate Media formulations are filtered before sterilization to remove the presence of any non-viable bacteria.

The formula of AnaeroGRO™ Thioglycollate with H and K is designed to prevent the harmful effects of toxic Reactive Oxygen Species (ROS) that may damage obligate anaerobes and inhibit the growth of more fastidious species.

FORMULA

Ingredients per liter of deionized water:*

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
Hemin	5.0mg
Vitamin K	1.0mg
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 (evaporation, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, oxygen exposure and freezing.

The expiration dating 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 quality control incubation times.

The plates must be inoculated **immediately** after opening the AnaeroGRO™ pouch. After inoculation, the plates must be placed **immediately** into an anaerobic atmosphere (pouch, jar, or chamber) to ensure optimal growth of anaerobic bacteria.

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 *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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired*

Infections: Approved Guideline.

Sterilize all biohazard waste before disposal.

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

Refer to the document SDS Search instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Method of Use: Consult the listed references for the appropriate cultivation techniques using this medium.[1,2,3,6,10,11] To avoid oxygen exposure, liquid specimens may be injected directly through the rubber septum of the hungate screw cap with a needle and syringe. Thioglycollate with H and K should be incubated at 35ºC, checking daily, as needed. When handling be careful not to agitate the broth which will introduce oxygen into the medium. Growth or turbidity should be confirmed by Gram stain and subcultured onto an appropriate growth medium, such as Brucella Agar with H and K (Cat. no. AG301). It is common for a whitish precipitate to form in this medium due to the agar content, however 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 organisms in this medium.[1,2,3,6,10,11]

LIMITATIONS

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

In test samples, the proper surface to volume ratio of the Thioglycollate with H and K 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 use Thioglycollate as the only method for growing anaerobes. Some anaerobes will not grow in Thioglycollate, even when enriched.

Plated media is required to isolate anaerobes from mixed cultures. This is best accomplished with the use of selective media such as BBE (Cat. no. AG051), LKV (Cat. no. AG601), and PEA (Cat. no. AG901). Rapidly growing organisms in a broth may overgrow the anaerobes, making recovery difficult.

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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

	Inoculation	Incubation			

Test Organisms	Method*	Time	Temperature	Atmosphere	Results
Clostridium perfringens ATCC® 13124***	A	24-48hr	35°C	Aerobic**	Growth
Bacteroides levii ATCC® 29147	A	24-48hr	35°C	Aerobic**	Growth
Bacteroides vulgatus ATCC® 8482	A	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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

AnaeroGRO™ Thioglycollate with H and K should appear translucent, and light amber in color. A white calcium carbonate chip should also be present in each tube.

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

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

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

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

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

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

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

11. Summanen, P., and E.J. Baron. 2002. *Wadsworth Anaerobic Bacteriology Manual*, 6th ed. Star Publishing Company, Belmont, CA.

12. Engelkirk, P.G., J. Duben-Engelkirk, and V.R. Dowell, Jr. 1992. *Principles and Practice of Clinical Anaerobic Bacteriology*. Star Publishing Company, Belmont, CA.

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

HDQA 2207A [C]