IFU

Instructions for Use

CRITERION™ MIDDLEBROOK 7H10 AGAR BASE

Cat. no. C6280	CRITERION™ Middlebrook 7H10 Agar Base	38gm
Cat. no. C6281	CRITERION™ Middlebrook 7H10 Agar Base	500gm
Cat. no. C6282	CRITERION™ Middlebrook 7H10 Agar Base	2kg
Cat. no. C6283	CRITERION™ Middlebrook 7H10 Agar Base	10kg
Cat. no. C6284	CRITERION™ Middlebrook 7H10 Agar Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Middlebrook 7H10 Agar Base is recommended for use in qualitative procedures for the isolation and cultivation of *Mycobacterium* species.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

SUMMARY

In 1947 Dubos and Middlebrook formulated a media (7H9) containing albumin and oleic acid which enhanced the growth of tubercle bacilli, and protected the organisms against a variety of toxic agents.[5] Later, in 1958, Middlebrook and Cohn improved this first formulation and developed a media (7H10) which allowed more luxuriant, faster growth of *Mycobacterium* species.[9]

CRITERION™ Middlebrook 7H10 Agar Base is a non-selective medium containing a variety of inorganic salts, sodium citrate, vitamins, co-factors, oleic acid, albumin, biotin, catalase and malachite green. The inorganic salts provide substances essential for the growth of mycobacteria. Sodium citrate, when converted to citric acid, holds the inorganic cations in solution. Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria. Biotin and catalase help stimulate the revival of damaged organism. Glycerol may be added to the base medium as a source of carbon and energy for the tubercle organisms. OADC Enrichment which must be added aseptically to the medium after autoclaving contains the following required additives: albumin to protect the tubercle bacilli against toxic agents; oleic acid, a fatty acid utilized in the metabolism of the organism; sodium chloride to maintain osmotic equilibrium; catalase to destroy any toxic peroxides in the medium; and dextrose as an energy source.

FORMULA

Gram weight per 900ml deionized water:	19.0gm
Monopotassium Phosphate	1.5gm
Disodium Phosphate	1.5gm

Ammonium Sulfate	0.5gm
L-Glutamic Acid	0.5gm
Sodium Citrate	0.4gm
Ferric Ammonium Citrate	40.0mg
Magnesium Sulfate	25.0mg
Copper Sulfate	1.0mg
Zinc Sulfate	1.0mg
Pyridoxine	1.0mg
Calcium Chloride	0.5mg
Biotin	0.5mg
Malachite Green	0.25mg
Agar	15.0gm
OADC Enrichment (sold separately) **	100.0ml

Final pH 6.6 +/- 0.2 at 25ºC.

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

** OADC Enrichment (sold separately - Cat. no. U98):

Bovine Albumin	5.0gm
Dextrose	2.0gm
Sodium Chloride	0.85gm
Beef Catalase	4.0mg
Oleic Acid	50.0mg

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light beige with a green tint.

Store the prepared culture media at 2-8ºC. **Protect from light.**

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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

Note: Hydrate culture media using 900ml of distilled or deionized water per liter of media. The OADC Enrichment added after sterilization will bring the volume to a full liter.

1. Suspend 19.0gm of the dehydrated culture media in 900ml of distilled or deionized water containing 5ml of glycerol.

2. Heat to boiling and mix to dissolve completely.

3. Sterilize in the autoclave at 121ºC. for 15 minutes.

4. Cool to 45-50ºC.

5. Aseptically add 100ml per liter of sterile OADC. Other enrichments may be added as desired.

6. Dispense into containers as desired.

7. **Protect media from light.**

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references or refer to the prepared media Instructions for Use (IFU) for Cat. No. W30.

LIMITATIONS

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

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results. Always take pH reading at room temperature.

Prepared Middlebrook 7H10 Agar requires incubation in a 5-10% CO_2 atmosphere in order to recover mycobacteria. For unknown reasons, mycobacteria are not recovered well from candle extinction jars.[7]

Keep inoculated media away from light or excessive heat, as exposure results in the release of formaldehyde in the media which may inhibit or kill mycobacteria.

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

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, as well as OADC Enrichment (Cat. no. U98), etc., 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	
Mycobacterium tuberculosis H37RV ATCC® 25177	G	21 days	35°C	CO_2**	Growth; colonies visible in 2 weeks, mature in 3 weeks
Mycobacterium kansasii Group I ATCC® 12478	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium scrofulaceum Group II ATCC® 19981	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium intracellulare Group III ATCC® 13950	G	21 days	35°C	CO_2**	Growth; colonies seen in 2 weeks, mature in 3 weeks
Mycobacterium fortuitum Group IV ATCC® 6841	G	21 days	35°C	CO_2**	Growth; colonies visible in 4 days

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

USER QUALITY CONTROL

Users of dehydrated 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. In addition, refer to the following document "Finished Product Quality Control Procedures," for more information on QC or see the reference(s) for more specific information.

PHYSICAL APPEARANCE

CRITERION™ Middlebrook 7H10 Agar Base powder should appear homogeneous, free-flowing, and light beige with a greenish tint in color. The prepared media should appear slightly opalescent, and light amber in color.

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. Cohn, M.L., et al. 1968. *Am. Rev. Respir. Dis.*; 98:295.

5. Dubos, R.J. and G. Middlebrook. 1947. *Am. Rev. Tuberc.*; 56:334-345.

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

7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

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

9. Middlebrook, G. and M.L. Cohn. 1958. *Am. J. Public Health*; 48:844-853.

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

11. Vestal, A.L. 1975. *Procedures for the isolation and identification of mycobacteria*. DHEW (CDC 75-8230). Centers for Diseases Control. Atlanta, GA.

12. Welch, D.F., et al. 1993. *Timely culture for mycobacteria which utilizes a microcolony method. J. Clin. Microbiol.*; 31: 2178-2184.

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

HDQA 2207B [D]