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Instructions for Use

CRITERION™ MIDDLEBROOK 7H9 BROTH BASE

Cat. no. C6301	CRITERION™ Middlebrook 7H9 Broth Base	500gm

INTENDED USE

Hardy Diagnostics CRITERION™ Middlebrook 7H9 Broth Base, supplemented with ADC Enrichment, is recommended for use in the cultivation of *Mycobacterium* spp. from sterile fluids. The medium can also be used for preparing dilutions of mycobacteria for antimicrobial testing.

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 growth medium known as 7H9, which contains albumin and oleic acid, to enhance the growth of tubercle bacilli; the medium also protects these microorganisms against a variety of toxic agents.[5] CRITERION™ Middlebrook 7H9 Broth Base is designed to be supplemented with Middlebrook ADC Supplement (Cat. no. X95). Middlebrook ADC Supplement provides nutrients necessary for mycobacterial growth and must be added aseptically to the medium after autoclaving.

Bovine albumin, dextrose, and catalase are components of Middlebrook ADC Enrichment. Albumin, which acts as a protective agent, binds free fatty acids that may be toxic to *Mycobacterium* spp. Dextrose serves as an energy source. Toxic peroxides that may be present in the medium are destroyed by catalase. Additionally, the basal medium contains glycerol, biotin and sodium citrate. Biotin helps to stimulate the revival of damaged cells as well as provides substrates needed for a variety of carboxylation and decarboxylation reactions. Sodium citrate, when converted to citric acid, holds inorganic cations in solution.

Along with the ADC Enrichment, additional supplements can be added to the medium, as needed: glycerol (2ml/L) to provide an abundant source of carbon and energy for tubercle organisms and polysorbate 80 (0.5ml/L) for improved growth. Prepared Middlebrook 7H9 Broth can also be used as a subculture medium for *Mycobacterium* species and for the preparation of inocula for drug susceptibility testing.

FORMULA*

Gram weight per 900ml deionized water:	4.7gm
Disodium Phosphate	2.5gm
Monopotassium Phosphate	1.0gm
L-Glutamic Acid	0.5gm
Ammonium Sulfate	0.5gm

Sodium Citrate	0.1gm
Magnesium Sulfate	50.0mg
Ferric Ammonium Citrate	40.0mg
Zinc Sulfate	1.0mg
Copper Sulfate	1.0mg
Pyridoxine	1.0mg
Calcium Chloride	0.5mg
Biotin	0.5mg
ADC Enrichment (Sold separately)**	100.0ml

pH before ADC Enrichment 6.6 +/- 0.1 at 25ºC.

Final pH with ADC Enrichment 6.8 +/- 0.3 at 25ºC.

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

** **Middlebrook ADC Enrichment** (sold separately - Cat. no. X95):

Bovine Albumin	5.0gm
Dextrose	2.0gm
Beef Catalase	3.0mg

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.

Store the prepared culture media at 2-8ºC.

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 ADC Enrichment added after sterilization will bring the volume to a full liter.

1. Suspend 4.7gm of the dehydrated culture media in 900ml of distilled or deionized water. Stir to mix thoroughly.

2. Heat as necessary 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 ADC (Cat. no. X95). Other enrichments may be added, as desired.

6. Dispense into containers as desired.

7. **Protect media from light.**

PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Consult listed references for information on specimen collection.[1-3,6,7,11]

Method of Use:

1. Middlebrook 7H9 Broth is used primarily for specimens from sterile body sites and for growth of pure cultures of mycobacteria for use in laboratory studies. Using aseptic techniques, inoculate a homogenized or centrifuged specimen directly to the medium. Consult listed references for methods.[1-3,6,7,11]

2. Incubate medium in a 5-10% CO_2 atmosphere at 35 +/- 2ºC., for up to eight weeks. Protect from light. Caps of tubes should be loosened for at least one week to allow circulation of CO_2. Tighten caps, thereafter, to prevent dehydration. Loosen caps briefly once a week in order to replenish CO_2.

3. Examine cultures within five to seven days after inoculation and weekly thereafter for up to eight weeks.

Mycobacterial growth from the broth can be used for additional laboratory test procedures as required. It is recommended that biochemical testing be done for complete identification.

Cultures from skin lesions suspected to be *M. marinum* or *M. ulcerans* should be incubated at 25-33ºC for primary incubation. Cultures suspected to contain *M. avium* or *M. xenopi* exhibit optimum growth at 40-42ºC and incubate a duplicate culture at 35-37ºC.[15]

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of *Mycobacterium* species on this medium.[1-3,6,7,11]

Turbidity at the bottom of or throughout the tube indicates growth.

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.

Middlebrook 7H9 media require 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]

Negative culture results do not rule out active mycobacteria infection.

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 loops, slides, decontamination supplies, applicator sticks, pipets, incinerators, CO_2 incubator, biological hoods, and microscopes, etc., as well as serological and biochemical reagents, such as sterile ADC Enrichment (Cat. no. X95), 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® 27294***	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube
Mycobacterium kansasii, Group I ATCC® 12478***	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube
Mycobacterium scrofulaceum , Group II ATCC® 19981***	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube
Mycobacterium intracellulare , Group III ATCC® 13950***	G	21 days	35°C	CO_2**	Growth; turbidity at bottom or throughout tube
Mycobacterium fortuitum, Group IV ATCC® 6841***	G	21 days	°35°C	CO_2**	Growth; turbidity at bottom or throughout tube

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

** Atmosphere of incubation is enriched with 5-10% CO_2.

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

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 7H9 Broth Base should appear homogeneous, free-flowing, and light beige in color. The prepared medium should appear clear, and colorless to 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.

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

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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 of the isolation and identification of mycobacteria.* DHEW (CDC 75-8230). Centers for Diseases Control. Atlanta, GA.

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15. Kent and Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. USDHHS. Centers for Disease Control, Atlanta, GA.

ATCC is a registered trademark of the American Type Culture Collection.

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