IFU

Instructions for Use

CRITERION™ MYCOBACTERIA 7H11 AGAR BASE

Cat. no. C6290	CRITERION™ Mycobacteria 7H11 Agar Base	38g
Cat. no. C6291	CRITERION™ Mycobacteria 7H11 Agar Base	500g
Cat. no. C6292	CRITERION™ Mycobacteria 7H11 Agar Base	2kg
Cat. no. C6293	CRITERION™ Mycobacteria 7H11 Agar Base	10kg

INTENDED USE

Hardy Diagnostics CRITERION™ Mycobacteria 7H11 Agar Base is recommended for the cultivation of *Mycobacterium* spp.

Dehydrated culture media is a raw material not intended for use in the diagnosis of human disease. For implementation, this product requires additional processing and supplementation of ingredients before use.

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] In 1958, Middlebrook and Cohn improved this formulation and developed a media (7H10) which allowed for more luxuriant and rapid growth of *Mycobacterium* species.[9] In 1968, Cohn incorporated casein hydrolysate into 7H10 medium to stimulate the growth of mycobacteria that would otherwise not grow on this medium. This formulation was then designated as 7H11 Agar, and is recommended over 7H10 Agar.[4,7]

Hardy Diagnostics CRITERION™ Mycobacteria 7H11 Agar Base contains inorganic compounds that supply essential growth stimulating inorganic salts, as well as vitamins and necessary co-factors. Sodium citrate is converted to citric acid, which holds inorganic cations in solution. Casein peptone is incorporated into 7H11 Agar as a growth stimulant for strains of drug resistant *Mycobacterium tuberculosis*.[2,8] Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria. Biotin helps stimulate the revival of damaged target organisms. It is also involved in a variety of carboxylation and decarboxylation reactions. During preparation, the medium should be supplemented with glycerol to provide a source of carbon and energy for the tubercle organisms. OADC Enrichment must also added during preparation and contains the following additives required for growth: 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 liter:	19.0g/L
Monopotassium Phosphate	1.5g

Disodium Phosphate	1.5g
Casein Peptone	1.0g
Ammonium Sulfate	0.5g
L-Glutamic Acid	0.5g
Sodium Citrate	0.5g
Magnesium Sulfate	50.0mg
Ferric Ammonium Citrate	40.0mg
Pyridoxine Hydrochloride	1.0mg
Biotin	0.5mg
Malachite Green	1.0mg
Agar	15.0g

Final pH 6.6 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) that contain dehydrated culture medium at 2-30ºC. Dehydrated culture medium is very hygroscopic and will clump when exposed to moisture and air. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. Dehydrated culture media should be discarded if clumped, if the media is not free-flowing or if the color has changed from its original light beige with green tint.

Store the prepared culture media at 2-8ºC and do not remove the container desiccant, if applicable.

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

1. Suspend 19.0g of the dehydrated culture media in 900ml of distilled or deionized water and 5ml of glycerol. Stir to mix thoroughly.

2. Boil to dissolve completely. Do not overheat.

3. Autoclave at 121ºC. for 10 minutes.

4. Cool to 45-50ºC.

5. Aseptically add 100ml of OADC Enrichment (Cat. no. U98). Mix well.

6. Protect the prepared 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.[1-3,6,7,13]

Method of Use:

1. Inoculate the agar with specimen, after decontamination and neutralization, according to test procedures recommended by the Centers for Disease Control (CDC). Consult listed references for methods.[1-3,6,7,13]

2. Incubate medium in a CO_2 atmosphere at 35-37°C. Protect from light.

3. Examine the media within five to seven days, and weekly thereafter for up to eight weeks.

4. Examine plates under light for the appearance of macroscopic growth.

5. Examine tubes under light and magnifying mirror for macroscopic growth. Record and describe colony morphology on the first day growth is observed.

6. Consult appropriate references for recording the number of colonies and for aid in the biochemical identification of acid-fast bacilli.[1,2,6,13]

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of *Mycobacterium* species on this medium.[1-3,6,7,13] Examine and record each type of colony morphology, pigment, and growth rate. Biochemical testing is required for definitive identification.

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.

Accurate counting may be difficult with spreading colonies.

Rare, fastidious microorganisms may not grow on selective media formulations.

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, incubators, tubes, bottles, petri dishes, glycerol, 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 H37Ra 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 visible in 2 weeks, mature in 3 weeks
Mycobacterium scrofulaceum Group II ATCC® 19981	G	21 days	35°C	CO_2**	Growth: colonies visible in 2 weeks, mature in 3 weeks
Mycobacterium intracellulare Group III ATCC® 13950	G	21 days	35°C	CO_2**	Growth: colonies visible 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.

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

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™ Mycobacteria 7H11 Agar Base powder should appear homogeneous, free-flowing, and light beige in color with a green tint. The prepared medium 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. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

3. Tille, P.M., 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. Mitchison, D.A., et al. 1972. *J. Med. Microbiol*; 5:165-175.

11. Mitchison, D.A., et al. 1973. *J. Clin. Pathol.*; 26:250-252.

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

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

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

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