

Instructions for Use

CRITERION™ EMB (EOSIN METHYLENE BLUE) AGAR, HHT (HOLT-HARRIS AND TEAGUE)

Cat. no. C7330	CRITERION™ EMB Agar, HHT	72gm
Cat. no. C7331	CRITERION™ EMB Agar, HHT	500gm
Cat. no. C7332	CRITERION™ EMB Agar, HHT	2kg
Cat. no. C7333	CRITERION™ EMB Agar, HHT	10kg
Cat. no. C7334	CRITERION™ EMB Agar, HHT	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ EMB Agar, HHT is recommended for the isolation and identification of gram-negative enteric bacteria.

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

Eosin Methylene Blue (EMB) Agar was originally developed by Holt-Harris and Teague.⁽¹⁾ Eosin dye was employed to inhibit the growth of gram-positive bacteria. Methylene blue was added as an indicator. Lactose and sucrose served as the nutrients. The production of acid, upon lactose- or sucrose-fermentation, resulted in the two dyes interacting to produce brown to blue-black colonies. This formulation gives sharp and distinct differentiation between colonies of lactose- and non-lactose-fermenting organisms. However, it does not discriminate between which carbohydrate (lactose or sucrose) is being fermented. *Yersinia enterocolitica*, which ferments sucrose but not lactose, will produce the same blue-black colony as lactose-fermenters.⁽²⁻⁶⁾ Gram-positive bacteria are inhibited by eosin Y.

FORMULA

Gram weight per liter:	36.0gm/L
Gelatin Peptone	10.0gm
Lactose	5.0gm
Sucrose	5.0gm
Dipotassium Phosphate	2.0gm
Eosin Y	0.4gm
Methylene Blue	65.0mg

Final pH 7.2 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30°C. 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 pinkish-purple.

Store the prepared culture media at 2-8°C.

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 36.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.
2. Heat to boiling to dissolve completely.
3. Sterilize in the autoclave at 121°C. for 15 minutes.
4. Cool to 45-50°C.

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. G263.

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.

Some gram-positive bacteria, such as enterococci, staphylococci and yeasts will grow on this medium and usually form pinpoint colonies.

Non-pathogenic, non-lactose-fermenting organisms will also grow on this medium.

Additional biochemical tests must be performed in order to distinguish these organisms from the pathogenic bacterial strains.

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, 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	
<i>Escherichia coli</i> ATCC® 25922	A	24hr	35°C	Aerobic	Growth; colonies blue-black with dark centers and green metallic sheen
<i>Salmonella enterica</i> ATCC® 14028	A	24hr	35°C	Aerobic	Growth; colonies colorless to amber
<i>Enterococcus faecalis</i> ATCC® 29212	B	24hr	35°C	Aerobic	Partial to complete inhibition; pinpoint colonies at 24 hours

* 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™ EMB Agar, HHT powder should appear homogeneous, free-flowing, and pinkish-purple in color. The prepared media should appear clear, usually with a fine precipitate evenly dispersed throughout, and purple with green-orange tinge in color.

REFERENCES

1. *J. Infectious Diseases*; 18:596, 1916.
2. 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.
3. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
4. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
6. MacConkey, A.T. 1905. Lactose-fermenting bacteria in faeces. *J. Hyg.*; 5:333-379.
7. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

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

IFU-10157[A]



1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: HardyDiagnostics.com

Email: TechnicalServices@HardyDiagnostics.com

[Ordering Information](#)

Distribution Centers:

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