



Instructions for Use

CRITERION™ BILE ESCULIN AGAR (BEA)

Cat. no. C5180	CRITERION™ Bile Esculin Agar (BEA)	124gm
Cat. no. C5181	CRITERION™ Bile Esculin Agar (BEA)	500gm
Cat. no. C5182	CRITERION™ Bile Esculin Agar (BEA)	2kg
Cat. no. C5183	CRITERION™ Bile Esculin Agar (BEA)	10kg
Cat. no. C5184	CRITERION™ Bile Esculin Agar (BEA)	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Bile Esculin Agar (BEA) is recommended for use as a differential medium in the isolation and presumptive identification of enterococci/group D streptococci.

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

Esculin hydrolysis was first described by Rochaix in 1924.⁽⁸⁾ Swan first introduced the use of Bile Esculin Agar in 1954.⁽⁹⁾ In 1970, Facklam and Moody determined that the use of the bile esculin test was a reliable way of identifying group D streptococci from non-group D streptococci.⁽³⁾ When using BEA in biochemical testing of group D streptococci, they found that all group D streptococci will blacken this medium.⁽³⁾ Other researchers have used BEA for the presumptive identification of *Enterobacter* spp., *Klebsiella* spp., and *Serratia* spp., among the Enterobacteriaceae.

This medium contains esculin, ferric citrate to provide ferric ions, and 4% oxbile to inhibit most other strains of non-group D streptococci. Esculin is hydrolyzed by group D streptococci to form dextrose and esculin. This compound reacts with the ferric ions contained within the medium, turning the medium from its original amber color to a dark brown to black. Thus the tolerance to the presence of bile and the hydrolysis of esculin provide the means to presumptively identify group D streptococci.

FORMULA

Gram weight per liter:	64.0gm/L
Oxbile (Oxgall)	40.0gm
Peptone	5.0gm
Beef Extract	3.0gm
Esculin	1.0gm

Ferric Citrate	0.5gm
Agar	15.0gm

Final pH 6.6 +/- 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 greenish-beige.

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

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.

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." 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.

METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 62.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.
2. Heat to boiling and mix to dissolve completely.
3. Sterilize in the autoclave at 121. for 15 minutes. **Heat sensitive.** Overheating may cause blackening of media.
4. Cool to 50-55°C. and aseptically add enrichments (50ml of filter-sterilized horse serum), if desired.

5. Dispense as desired.

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. G12.

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.

Some strains of *Staphylococcus*, *Aerococcus*, and *Listeria monocytogenes* may grow in the presence of bile and hydrolyze esculin. *L. monocytogenes* will form minute black colonies.

A heavy inoculum on BEA may cause interpretation of the bile esculin test difficult to read. Excess inoculum decreases the ability of the bile to inhibit growth of other gram-positive organisms that may hydrolyze esculin.

There are a few streptococci that do not hydrolyze esculin but will grow in the presence of bile. Growth without blackening of this medium does not constitute a positive test.

BEA does not contain azide; as a result, gram-negative rods will grow on this medium. Many of these organisms may hydrolyze esculin.

Occasional viridans strains testing *Escherichia coli* on Bile Esculin Agar or will display reactions that are difficult to interpret. Of the viridans group, 5 to 10% may be able to hydrolyze esculin in the presence of bile.⁽⁴⁾

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

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Enterococcus faecalis</i> ATCC® 29212	A	18-48hr	35°C	Aerobic	Growth; blackening of media around colonies
<i>Streptococcus pyogenes</i> ATCC® 19615	B	18-48hr	35°C	Aerobic	Partial to complete inhibition

* 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 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

CRITERION™ Bile Esculin Agar (BEA) powder should appear homogeneous, free-flowing, and greenish-beige in color. The prepared media should appear slightly opalescent with a bluish-tinge, and greenish to medium 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. Facklam, R.R. and M.D. Moody. 1970. *Appl. Microbiol.*; 20:245.
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. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
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8. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
9. Rochaix. 1924. *Cr. Soc. Biol.*, Paris; 90:771.
10. Swan, A. 1954. *J. Clin. Path.*; 7:160-163.

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

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