IFU

Instructions for Use

CRITERION™ BILE ESCULIN AZIDE MEDIA

Cat. no. C5170	CRITERION™ Bile Esculin Azide Agar	115.6gm
Cat. no. C5171	CRITERION™ Bile Esculin Azide Agar	500gm
Cat. no. C5172	CRITERION™ Bile Esculin Azide Agar	2kg
Cat. no. C5173	CRITERION™ Bile Esculin Azide Agar	10kg
Cat. no. C5174	CRITERION™ Bile Esculin Azide Agar	50kg
Cat. no. C5190	CRITERION™ Bile Esculin Azide Broth	86gm
Cat. no. C5191	CRITERION™ Bile Esculin Azide Broth	500gm
Cat. no. C5192	CRITERION™ Bile Esculin Azide Broth	2kg
Cat. no. C5193	CRITERION™ Bile Esculin Azide Broth	10kg
Cat. no. C5194	CRITERION™ Bile Esculin Azide Broth	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Bile Esculin Azide Media are recommended for the isolation and differentiation of group D streptococci from non-group D.

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] Facklam and Moody, in 1970, 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*, *Klebsiella* and *Serratia*.

This medium contains esculin, ferric citrate to provide ferric ions, and 1% oxbile to inhibit most other strains of non-group D streptococci. Sodium azide is incorporated into the medium to inhibit gram-negative bacteria. Esculin is hydrolyzed by group D streptococci to form dextrose and esculetin. This compound reacts with the ferric ions contained within the medium, turning the medium 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

Bile Esculin Azide Agar:

Gram weight per liter:	61.7gm/L
Pancreatic Digest of Casein	20.0gm
Oxbile (Oxgall)	10.0gm
Yeast Enriched Meat Peptone	10.0gm
Sodium Chloride	5.0gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Sodium Azide	0.15gm
Agar	15.0gm

Final pH 7.1 +/- 0.2 at 25ºC.

Bile Esculin Azide Broth:	
Gram weight per liter:	43.0gm/L
Pancreatic Digest of Casein	16.0gm
Oxbile (Oxgall)	10.0gm
Yeast Enriched Meat Peptone	9.5gm
Sodium Chloride	5.0gm
Sodium Citrate	1.0gm
Esculin	1.0gm
Ferric Ammonium Citrate	0.5gm
Sodium Azide	0.25gm

Final pH 7.1 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30ºC. 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 tan.

Store the prepared culture media at 2-30ºC.

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

Bile Esculin Azide Agar:

1. Suspend 57.8gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling and mix to dissolve completely. Avoid overheating.

3. Sterilize in the autoclave at 121ºC. for 15 minutes. Overheating may cause the media to darken.

4. Cool to 50-55ºC and dispense into sterile petri dishes. If tubed, allow the medium to solidify in the slanted position.

Bile Esculin Azide Broth:

1. Suspend 43.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.

2. Heat as necessary to dissolve completely. Avoid overheating.

3. Dispense into autoclavable tubes.

4. Sterilize in the autoclave at 121ºC. for 15 minutes. Overheating may cause the media to darken.

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. G11 (agar) and K11 (broth).

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	
Bile Esculin Azide Agar and Bile Esculin Azide Broth:					
Enterococcus faecalis ATCC® 29212	A	24-48hr	35°C	Aerobic	Growth; blackening of media around colonies
Streptococcus pyogenes ATCC® 19615	B	24-48hr	35°C	Aerobic	Partial to complete inhibition
Escherichia coli ATCC® 25922	B	24-48hr	35°C	Aerobic	Partial to complete inhibition; small colorless colonies

* 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™ Bile Esculin Azide Agar powder should appear homogeneous, free-flowing, and tan in color. CRITERION™ Bile Esculin Azide Broth powder should appear homogeneous, free-flowing, and tan in color. The prepared media should appear clear, and medium to dark amber, with a slight bluish-tinge in color. The agar may be slightly opalescent.

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. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. Facklam, R.R. and M.D. Moody. 1970. *Appl. Microbiol.*; 20:245.

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

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

6. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

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

8. Rochaix. 1924. *Cr. Soc. Biol.*, Paris; 90:771.

9. Swan, A. 1954. *J. Clin. Path.*; 7:160-163.

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

IFU-10122[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:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

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

HDQA 2207B [D]