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Instructions for Use

CRITERION™ AZIDE BLOOD AGAR BASE

Cat. no. C5040	CRITERION™ Azide Blood Agar Base	66gm
Cat. no. C5041	CRITERION™ Azide Blood Agar Base	500gm
Cat. no. C5042	CRITERION™ Azide Blood Agar Base	2kg
Cat. no. C5043	CRITERION™ Azide Blood Agar Base	10kg
Cat. no. C5044	CRITERION™ Azide Blood Agar Base	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Azide Blood Agar Base is used for isolation of streptococci and staphylococci. Blood may be added for the determination of hemolytic reactions.

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

CRITERION™ Azide Blood Agar Base is used for the isolation of gram-positive organisms from both clinical and non-clinical specimens. Edwards, in 1933, used a selective broth containing crystal violet and sodium azide in the isolation of mastitis streptococci.[1] Snyder and Lichstein added 0.01% sodium azide to prevent the swarming of *Proteus* species and also permitted the isolation of streptococci from mixed bacterial populations.[3,7] Edwards' medium was modified by Packer by preparing Infusion Blood Agar with 1:15,000 sodium azide and 1:500,000 crystal violet for the study of bovine mastitis.[6] Mallmann, Botwright and Churchill reported there was a bacteriostatic effect on gram-negative bacteria when sodium azide is present.[4] The Azide Blood Agar Base formula was based on the work of these researchers.

Beef extract and tryptose serve as sources of carbon, nitrogen, vitamins and minerals. Sodium chloride is added to maintain the osmotic equilibrium. Sodium azide inhibits cytochrome oxidase in gram-negative bacteria. CRITERION™ Azide Blood Agar Base can be supplemented with 5-10% sheep, rabbit, or horse blood for the isolation, cultivation and determination of hemolytic reactions of fastidious pathogens.

FORMULA*

Gram weight per liter:	33.0gm/L
Casein Peptone	5.0gm
Meat Peptone	5.0gm
Sodium Chloride	5.0gm

Beef Extract	3.0gm
Sodium Azide	0.2gm
Agar	15.0gm

Final pH 7.2 +/- 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. Protect dehydrated culture media from moisture and light. Keep container tightly closed, dehydrated medium is very hygroscopic. The dehydrated culture media should be discarded if it is not free flowing and homogenous or if the color has changed from its original tan color.

Store the prepared 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

1. Suspend 33.0gm of the dehydrated culture media in 1 liter of distilled or deionized water.

2. Heat to boiling to dissolve completely.

3. Autoclave at 121ºC. for 15 minutes.

4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50ºC. Mix well.

PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

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.

Hemolytic patterns of streptococci grown on Azide Blood Agar are somewhat different than those seen on ordinary blood agar. The sodium azide enhances hemolysis.[6]

Hemolytic patterns may vary with the source of animal blood or base medium used.[5]

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, 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	
Staphylococcus aureus ATCC® 25923	A	24-48hr	35°C	Aerobic	Growth; beta-hemolysis
Streptococcus pneumoniae ATCC® 6305	A	24-48hr	35°C	Aerobic	Growth; alpha-hemolysis
Streptococcus pyogenes ATCC® 19615	A	24-48hr	35°C	Aerobic	Growth; beta-hemolysis
Escherichia coli ATCC® 25922	B	24-48hr	35°C	Aerobic	Inhibited

* 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™ Azide Blood Agar Base powder should appear homogeneous, free-flowing and tan in color. The prepared media should appear slightly opalescent without precipitate, and light to medium amber in color. When prepared with 5% sheep blood the media should appear opaque, and cherry red in color.

REFERENCES

1. Edwards, S.J. 1933. The diagnosis of *Streptococcus mastitis* by cultural methods. *J. Comp. Pathol. Ther.*; 46:211.

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

3. Lichstein, H.C. and M.L. Snyder. 1941. The inhibition of the spreading growth of *Proteus* and other bacteria to permit the isolation of associated streptococci. *J. Bacteriol.*; 42:653.

4. Mallmann, Botwright, and Churchill. 1943. *J. Bacteriol.*; 46:343.

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

6. Packer, R.A. 1943. The use of sodium azide (NaN_3) as an inhibition substance of gram-negative bacteria. *J. Infect. Dis.*; 67:113.

7. Snyder, M.L. and H.C. Lichstein. 1940. Sodium azide as an inhibition substance of gram-negative bacteria. *J. Infect. Dis.*; 67:113.

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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]