IFU

Instructions for Use

CRITERION™ NITRATE BROTH

Cat. no. C6450	CRITERION™ Nitrate Broth	18gm
Cat. no. C6451	CRITERION™ Nitrate Broth	500gm
Cat. no. C6452	CRITERION™ Nitrate Broth	2kg
Cat. no. C6453	CRITERION™ Nitrate Broth	10kg
Cat. no. C6454	CRITERION™ Nitrate Broth	50kg

INTENDED USE

Hardy Diagnostics CRITERION™ Nitrate Broth is recommended for differentiating microorganisms based upon nitrate reduction.

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

The ability of bacteria to reduce nitrate is an important biochemical characteristic which aids in the identification of many microorganisms, particularly members of the family Enterobacteriaceae, and members of the *Haemophilus*, *Neisseria*, and *Branhamella* genera.[2,3,5,6]

Organisms which possess the enzyme nitroreductase vary in their ability to reduce nitrate. Some microorganisms reduce nitrate to nitrite while others further reduce the nitrate to form other end products such as ammonia, nitrogen gas, hydroxylamine, etc. The end product of nitrate reduction is dependent upon the bacterial species.[7]

The reduction of nitrate to nitrite is determined by the development of a red color complex upon the addition of sulfanilic acid solution (Nitrate Reagent A, Cat. no. Z71) and N, N-dimethyl-1-naphthylamine (Nitrate Reagent B, Cat. no. Z72). The sulfanilic acid reacts with nitrite to form a diazonium salt which then couples with N, N-dimethyl-1-naphthylamine to produce a red-dye complex. Absence of a red color reaction indicates that the organism has further reduced the nitrites to ammonia or nitrogen gas, or that unreduced nitrate is present, thus indicating the organism does not possess the nitroreductase enzyme.

If an organism does not possess the enzyme, nitrate will remain present in the medium. Application of zinc dust (Nitrate Reagent C, Cat. no. Z73) will convert nitrate to nitrite to form a red-dye complex. This test reaction is considered negative for nitrate reduction. If, however, the organism has reduced nitrate beyond nitrite to nitrogen gas, application of zinc dust will not produce a color change. The test is then considered positive for nitrate reduction.

FORMULA

Gram weight per liter:	9.0gm/L

Pancreatic Digest of Gelatin	5.0gm
Beef Extract	3.0gm
Potassium Nitrate	1.0gm

Final pH 6.9 +/- 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 light beige.

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

1. Suspend 9.0gm of the dehydrated culture media in 1 liter of distilled or deionized water until evenly dispersed.

2. Heat as necessary to dissolve completely.

3. Distribute into test tubes (with durham tubes if desired) and sterilize in the autoclave at 121ºC. for 15 minutes.

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. K42.

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.

Test isolates must be in pure culture and 18-24 hours old.

Interpretation of color reactions should be made immediately, as color reactions with a positive test may fade rapidly.

If air bubbles are present in the durham tube prior to inoculation, the tube should be inverted until the air is released from the durham tube. Failure to remove air bubbles prior to inoculation may result in reading the result as a false-positive reaction for gas reduction.

A faint pink color may be produced following addition of the nitrate reagents. This is not a positive result. Development of a **deep red** color is indicative of a positive reaction.

A negative zinc reduction (no color change) test, in combination with a negative nitrite reaction, is presumptive indication that the nitrate was reduced beyond the nitrite stage. Although a very common end product of nitrite reduction is nitrogen gas, other end products may be formed. Additional testing may be required to determine the final end products of the reaction.

To avoid false-negative nitrite reduction reactions, negative nitrite reactions must be verified by the addition of Nitrate Reagent C (zinc dust) to the medium.

Excess zinc dust has been reported to cause false-positive nitrite reduction reactions due to complete reduction of previously unreduced nitrate to ammonia.

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	**Reaction**
Escherichia coli ATCC® 25922	Positive nitrate reduction; deep red color seen after the addition of Reagents A and B
Acinetobacter baumannii ATCC® 19606	Negative nitrate reduction; no color change seen after the addition of Reagents A and B **and** red color forms after addition of Reagent C

* 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™ Nitrate Broth powder should appear homogeneous, free-flowing, and light beige in color. The prepared media should appear clear, 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. Ewing, W.H. 1986. *Edwards and Ewing's Identification of Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York.

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. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

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

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The Hardy Diagnostics manufacturing facility and quality

management system is certified to ISO 13485.