IFU

Instructions for Use

NITRATE BROTH WITH DURHAM TUBE

Cat. no. K42	Nitrate Broth with Durham Tube, 13x100mm Tube, 6ml	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Nitrate Broth with Durham Tube is recommended for use in the detection of nitrate reduction by bacteria.

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

Ingredients per liter of deionized water:*

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

Storage: Upon receipt store at 2-30ºC. Media should not be used if there are any signs of contamination, deterioration or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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.

PROCEDURE

Specimen Collection: This medium is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organisms. This product is used in conjunction with other biochemical tests to identify cultures of isolated organisms.

Method of Use:

1. Prior to inoculation, allow the medium to equilibrate to room temperature.

2. Using a loopful of inoculum from an 18-24 hour pure culture, inoculate the broth. A very heavy inoculum is recommended for organisms that do not grow well in the medium (such as some *Neisseria* spp.). See the "Limitations" section for more information.

3. Incubate the tube with loose cap at 35-37. in an aerobic atmosphere for 24-48 hours.

4. Observe for growth and the presence of gas bubbles in the durham tube. If gas is present and the organism is known to be a non-fermenter, the test is considered positive for nitrate reduction. If growth is apparent but no gas is present, or if gas is present and the organism is known to be a **fermenter** , proceed with the following steps:

5. Add five drops of Nitrate Reagent A (Cat. no. Z71) and five drops of Nitrate Reagent B (Cat. no. Z72) to the broth. **Note:** add reagents in the order listed.

6. Gently shake tube to mix reagents.

7. Observe for the development of a deep red color within two minutes following application of the reagents.

8. If a red color does not result after step 6 above, add approximately 6.0mg of Nitrate Reagent C (Cat. no. Z73) to the medium.

9. Observe for the development of a red color within 5-10 minutes following the addition of Nitrate Reagent C. A red color indicates that nitrate was **not** reduced. Absence of a red color reaction indicates nitrate reduction beyond nitrite.

INTERPRETATION OF RESULTS

A positive nitrate reduction test is indicated by the development of a deep red color after the addition of Nitrate Reagents A and B **or** no development of color after the addition of Nitrate Reagent C.

A negative nitrate reduction test is indicated by the absence of a deep red color complex after the addition of Nitrate Reagents A and B **and** formation of a red color complex after the addition of Nitrate Reagent C.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

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

A very heavy inoculum is recommended for organisms that do not grow well in the medium (such as some *Neisseria* spp.). The heavy inoculum will provide sufficient preformed enzymes for the reaction to occur.

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 loops, slides, staining supplies, Nitrate Reagents A, B and C, other culture media, microscopes, incinerators, incubators, etc., as well as serological and biochemical reagents, 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

End users of commercially prepared 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. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Nitrate Broth with Durham Tube should appear clear, and light amber in color.

Escherichia coli (ATCC® 25922) grown in Nitrate Broth with Durham Tube (Cat. no. K42). Incubated aerobically for 24 hours at 35ºC. Five drops of Nitrate Reagent A (Cat. no. Z71) and five drops of Nitrate Reagent B (Cat. no. Z72) were added subsequent to incubation. The red color development was indicative of a positive reaction: the reduction of nitrate to nitrite.

Acinetobacter baumannii (ATCC® 19606) grown in Nitrate Broth with Durham Tube (Cat. no. K42). Incubated aerobically for 24 hours at 35ºC. Five drops of Nitrate Reagent A (Cat. no. Z71) and five drops of Nitrate Reagent B (Cat. no. Z72) were added subsequent to incubation. No red color development indicated that nitrate was not reduced to nitrite (presumptive negative). To ensure nitrate was not reduced to an end product other than nitrite, all negatives should be confirmed by adding Nitrate Reagent C (Cat. no. Z73). A red color development is indicative of a "true negative" for nitrate reduction.

Showing confirmation reaction for *Acinetobacter baumannii* (ATCC® 19606). Since there was not red color development after the addition of Nitrate Reagents A and B, a small amount of Nitrate Reagent C, zinc dust (Cat. no. Z73) was added using the end of a sterile wooden stick. The red color development was indicative as a "true negative" for nitrate reduction.

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

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

5. Ewing, W.H. 1986. *Edwards and Ewing's Identification of Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York.

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

7. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*,, Lipincott Williams & Wilkins, Philadelphia, PA.

IFU-10610[B]

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 2207F [D]