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Instructions for Use

HARDYDISK™ NITRATE

Cat. no. Z7071	HardyDisk™ Nitrate	50 disks/cartridge

INTENDED USE

Hardy Diagnostics HardyDisks™ Nitrate are for the differentiation of anaerobes based on the ability to reduce nitrate.

SUMMARY

Organisms that possess the enzyme nitrate reductase are able to utilize nitrogen as a terminal acceptor in their electron transport chain. In this reaction, nitrate is reduced to nitrite, which may then be further reduced to nitrogen gas or ammonias. The HardyDisk™ Nitrate detects the nitrite that these organisms produce by the colored end product that is formed after addition of nitrate reagents.

Nitrate, when combined with sulfanilic acid and N,N-dimethyl-alpha-naphthylamine forms a red compound, p-sulfobenzene-azo-napthylamine, which is indicative of a positive reaction. If there is no color production (a negative reaction) zinc dust is added to confirm the negative reaction. Zinc will reduce residual nitrate to nitrite producing a red color (a confirmed negative reaction). If both the nitrate and zinc reactions are negative, the nitrate has been broken down beyond nitrite (a positive reaction for nitrate reduction).

FORMULA

HardyDisks™ Nitrate are impregnated with potassium nitrate and sodium molybdate. The disks are imprinted with the letters NRT.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20-8ºC. away from direct light. Disks should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Product is light and temperature sensitive. Protect from heat.

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

1. Streak a reduced blood agar plate to obtain confluent growth.

2. Aseptically place a Nitrate HardyDisk™ on the plate and press it to the agar with a sterile pair of forceps.

3. Incubate the plate, inverted, for 24-48 hours at 35ºC.

4. After the plates have been incubated, add one drop of Sulfanilic Acid (Nitrate Reagent A, Cat. no. Z71) and one drop of N,N-dimethyl-alpha-naphthylamine (Nitrate Reagent B, Cat. no. Z72).

5. Observe for a color change within 3-5 minutes.

6. If there is no color development, add a small amount of Nitrate Reagent C, Cat. no. Z73 (zinc dust), and examine for a red color change within 10 minutes.

INTERPRETATION OF RESULTS

A red color development after addition of Nitrate Reagents A and B is a positive test for the reduction of nitrate. If no color develops after addition of the reagents, the test is presumptively negative. Development of a red color after the addition of the Nitrate Reagent C is a confirmed negative reaction. When nitrate is reduced beyond nitrite, no color change occurs after the addition of the Nitrate Reagent C. This is considered a positive test for nitrate reduction.

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.

Nitrate HardyDisks™ are intended to aid in the identification of anaerobic bacteria. Additional biochemical and serological tests may be required for complete identification. See listed references for more information.[1-4]

Rapidly growing organisms may discolor Nitrate HardyDisks™, making interpretation of the color reaction difficult. In this case another method for determining nitrate reductase activity, such as the tube nitrate test, is recommended.[5]

Organisms that do not grow sufficiently may fail to produce enough nitrate reductase to visualize a positive reaction, giving a false-negative test result.

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, other culture media, swabs, applicator sticks,

incinerators, incubators, Nitrate Reagent A (Cat. no. Z71) and Nitrate Reagent B (Cat. no. Z72), and Nitrate Reagent C (Cat. no. Z73), etc., as well as other 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	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Propionibacterium acnes ATCC® 11827	B	24-48hr	35°C	Anaerobic	Positive; Red color development after the addition of Nitrate Reagents A and B
Clostridium difficile ATCC® 9689	B	24-48hr	35°C	Anaerobic	Negative; Red color development only after the addition of Reagent C (zinc dust)

* 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

HardyDisks™ Nitrate should appear as 1/4 inch filter paper disks imprinted with the letters NRT, and should appear white in color.

Showing a positive nitrate reduction reaction.
Propionibacterium acnes (ATCC® 11827) colonies growing on Brucella Agar with H and K (Cat. no. A30) with a Nitrate HardyDisk™ (Cat. no. Z7071) aseptically placed on the plate prior to anaerobic incubation (24 hours at 35 deg. C.). One drop of Nitrate Reagent A (Cat. no. Z71) and one drop of Nitrate Reagent B (Cat. no. Z72) were dropped onto the disk. The red color development was indicative of a positive reaction.

Showing a negative nitrate reduction reaction.
Clostridium difficile (ATCC® 9689) colonies growing on Brucella Agar with H and K (Cat. no. A30) with a Nitrate HardyDisk™ (Cat. no. Z7071) aseptically placed on the plate prior to anaerobic incubation (24 hours at 35 deg. C.). One drop of Nitrate Reagent A (Cat. no. Z71) and one drop of Nitrate Reagent B (Cat. no. Z72) were dropped onto the disk. The absence of a red color development was indicative of a negative reaction.

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology,* 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. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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

5. Wideman, P.A., et al. 1977. Simple disk technique for detection of nitrate reduction by anaerobic bacteria. *J. Clin. Micro.*; 5:315-319.

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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 2207F [D]