

Instructions for Use

ONPG DIFFERENTIATION DISKS

Cat. no. Z7321	ONPG Differentiation Disk	50 disks/cartridge
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INTENDED USE

HardyDisk™ ONPG Differentiation Disk is used to differentiate members of the Enterobacteriaceae and other microorganisms based on beta-galactosidase activity.

SUMMARY

Lactose is a disaccharide composed of molecules of galactose and glucose. The ability of bacteria to ferment lactose depends on two enzymes; permease and beta-galactosidase. Permease regulates the movement of lactose across the bacterial cell wall. Once lactose is inside the cell, it is broken down into the individual components, glucose and galactose, by beta-galactosidase.⁽¹⁾

However, some organisms lack permease and consequently appear as late or non-lactose-fermenters. The ONPG test is valuable for the detection of beta-galactosidase activity in late lactose-fermenting organism like *Shigella sonnei* and some strains of *Escherichia coli*.⁽⁶⁾ The ONPG test detects the enzyme beta-galactosidase with greater speed and sensitively than lactose-fermentation tests.

O-nitrophenyl-beta-D-galactopyranoside (ONPG) is an artificial substrate structurally similar to lactose with the exception that glucose is substituted with an o-nitrophenyl group. ONPG is able to enter the bacterial cell more easily than lactose as it is not dependent on the presence of the permease enzyme. If the organism possesses beta-galactosidase, the enzyme will split the beta-galactoside bond, releasing o-nitrophenol, which is a yellow-colored compound. The activity of the galactosidase enzyme is increased in the presence of sodium ions.⁽⁶⁾

The organism to be tested is taken from a medium containing a high concentration of lactose. A dense suspension (turbidity equivalent to a McFarland 3) is prepared. An ONPG disk is added to 0.5ml of the suspension. If the organism possesses beta-galactosidase, the enzyme will split the beta-galactoside bond, creating a yellow color change in the suspension. Organisms with strong beta-galactosidase activity can produce a positive reaction a few minutes after inoculation of the ONPG medium; other organisms may take up to 24 hours.

FORMULA

Each HardyDisk™ ONPG Differentiation Disk is prepared by impregnating carefully controlled concentrations of ONPG onto a 0.25 inch diameter filter paper disk.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at -20 to +8°C. away from direct light. Product should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Protect the product from light, excessive heat, and moisture.

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

1. Use a loop to transfer bacteria from pure 18-24 hour culture to a test tube containing 0.5ml of 0.85% sterile saline. The resulting suspension should be approximately equivalent in density to a McFarland 3 opacity standard.
2. Add a single ONPG disk to the dense bacterial suspension. Upon the addition of the disk the bacterial suspension will be clear.
3. Incubate at 37°C. and check hourly, for up to 4 hours, for the development of a yellow color change.
4. Incubate any negative reactions (colorless) for 24 hours. Observe after 24 hours for possible delayed reactions of late lactose-fermenters.

INTERPRETATION OF RESULTS

A yellow color change is a positive reaction; indicating beta-galactosidase activity. No color change is a negative reaction; indicating the absence of beta-galactosidase activity.

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.

All organisms to be tested must be inoculated from a lactose-containing medium (e.g., TSI, KIA, or MacConkey Agar).

Cultures that naturally produce a yellow pigment cannot be tested with this media.

The ONPG test is not a substitute for the determination of lactose-fermentation since only the beta-galactosidase enzyme is evaluated.⁽⁶⁾

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, and 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	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ATCC® 25922	D	1-24hr	35°C	Aerobic	ONPG positive; yellow color develops within 1-24 hours
<i>Proteus mirabilis</i> ATCC® 12453	D	24hr	35°C	Aerobic	ONPG negative; no color development in 24 hours

* 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

HardyDisk™ ONPG Differentiation Disks are 0.25 inch (in diameter) filter paper disks imprinted with the letters ONPG on both sides and should appear white in color.



Escherichia coli (ATCC® 25922) suspension in 0.85% sterile saline with a HardyDisk™ ONPG Differentiation Disks (Cat. no. Z7321). Incubated aerobically for one hour at 35°C. The yellow color development was indicative of a positive ONPG reaction.



Proteus mirabilis (ATCC® 12453) suspension in 0.85% sterile saline with a HardyDisk™ ONPG Differentiation Disks (Cat. no. Z7321). Incubated aerobically for 24 hours at 35°C. No yellow color development was indicative of a negative ONPG reaction.

REFERENCES

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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. Lowe, G.H. 1962. The rapid detection of lactose fermentation in paracolon organisms by the demonstrations of B-D-galactosidase. *Journal of Medical Lab Technology*; 19:21-25.
8. *Commission on Laboratory Accreditation, Laboratory Accreditation Program Microbiology Checklist*. College of American Pathologists. Rev. 9/30/2004.
9. Centers for Medicare and Medicaid, *Appendix C, Survey Procedures and Interpretive Guidelines for Laboratories and Laboratory Services*. Subpart K - Quality System for Non-Waived Testing. 493;1200-1265. www.cms.hhs.gov/clia.

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

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[Ordering Information](#)

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