

# Instructions for Use

## OXIDROP™

<a href="#">Cat. no. Z19</a>	Oxidase Powder, 10mL amber tube, 0.1gm	20 tubes/box
<a href="#">Cat. no. Z119</a>	OxiDrop™, Oxidase Test, 0.5oz. Polyethylene Bottle with Dropper Tip, 7.0ml	1 each

## INTENDED USE

Hardy Diagnostics OxiDrop™, Oxidase Test is a liquid ready-to-use reagent that is used in procedures to detect cytochrome oxidase activity in bacteria. Oxidase Powder can be used to prepare the oxidase reagent.

## SUMMARY

Cytochrome containing organisms produce an intracellular oxidase enzyme. This oxidase enzyme catalyzes the oxidation of cytochrome c. Organisms which contain cytochrome c as part of their respiratory chain are oxidase-positive and turn the reagent blue/purple. Organisms lacking cytochrome c as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the limits of the test, and are oxidase-negative.

## REAGENT FORMULA\*

N,N,N',N'-tetramethyl-p-phenylenediamine Dihydrochloride	12.0gm
Distilled Water	1000.0ml
Stabilizing Agent	

\* Adjusted and/or supplemented as required to meet performance criteria.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store Cat. no. Z119 at 2-8°C and Cat. no. Z19 at 2-30°C away from direct light. Product should not be used if there are any signs of deterioration, discoloration, contamination, 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

For. Cat. no. Z19: Add 10mL deionized water to one tube of oxidase powder containing 0.1gm of oxidase powder. Use the prepared oxidase reagent by following the procedure for Cat. no. Z119 below.

For. Cat. no. Z119: Use only 18-24 hour old colonies from non-selective, non-differential media.

Place a piece of filter paper in a sterile, plastic, disposable petri dish. Moisten the piece of filter paper with a few drops of oxidase reagent. Rub a small amount of the colony to be tested onto the paper using a platinum loop or a wooden applicator stick. Observe for blue-black color development.

Alternatively, a drop of the reagent can be placed directly on a colony on an agar plate, looking for the same color development described above.<sup>(2)</sup>

## INTERPRETATION OF RESULTS

Development of a blue-black color within 10-20 seconds indicates that the organism is positive for the presence of cytochrome oxidase.

**Important:** Any color change after 20 seconds should be disregarded.

## LIMITATIONS

The oxidase test can be used in the presumptive identification of *Neisseria* spp. and in the differentiation and identification of gram-negative bacilli. Oxidase-positive organisms should be examined by gram stain to determine morphology and gram reaction. Additional biochemical tests are recommended for complete identification.

Use of a nichrome or other iron containing loop may yield false-positive reactions. Platinum loops are recommended.

Most *Haemophilus* spp. are oxidase-positive. Less sensitive strips or reagents may yield false-negative results. Consult listed references for more information.<sup>(7)</sup>

Oxidase reactions of gram-negative bacilli should be determined on non-selective and non-differential media to ensure valid results. Also, colonies taken from media containing high levels of glucose may give false-negative reactions.<sup>(5)</sup>

It is recommended to use colonies that are 18-24 hours old. Older colonies will produce weaker reactions.

Any color changes appearing after 20 seconds should be disregarded.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies such as filter paper, empty petri dishes, platinum loops or wooden applicator sticks, and culture media, 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
<i>Pseudomonas aeruginosa</i> ATCC® 27853	Oxidase-positive; blue/purple color develops within 10-20 seconds
<i>Escherichia coli</i> ATCC® 25922	Oxidase-negative; no color development within 10-20 seconds

## USER QUALITY CONTROL

It is recommended that each new lot or shipment of reagent be tested with known positive and negative controls.<sup>(3,8)</sup>

## PHYSICAL APPEARANCE

Oxidase Powder should appear beige in color. OxiDrop™, Oxidase Test should appear clear, and slightly brown in color.



*Pseudomonas aeruginosa* (ATCC® 27853) applied to filter paper moistened with OxiDrop™ (Cat. no. Z119) reagent. The development of a blue/purple color within 10-20 seconds was indicative of a positive oxidase test.



*Escherichia coli* (ATCC® 25922) applied to filter paper moistened with OxiDrop™ (Cat. no. Z119) reagent. No development of a blue/purple color within 10-20 seconds was indicative of a negative oxidase test.

## 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. Centers for Medicare and Medicaid, *Appendix C, Survey Procedures and Interpretive Guidelines for Laboratories*

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

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

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

7. Holt, J.G. and N.R. Krieg. 1984. *Bergey's Manual of Systematic Bacteriology*, Vol. I. Williams & Wilkins, Baltimore, MD.

8. *Commission on Laboratory Accreditation, Laboratory Accreditation Program Microbiology Checklist*. College of American Pathologists. Rev. 9/30/2004.

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

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[Ordering Information](#)

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