



## OXIDASE TEST REAGENT BIOCHEMICAL IDENTIFICATION

**PRODUCT:****Tube:**

Oxidase Reagent (powder), item no. R6630  
Oxidase Reagent (liquid), item no. R6632

**PURPOSE:**

Oxidase Reagent is used to test for cytochrome oxidase activity in microorganisms. It is particularly useful in differentiating bacteria in the family *Enterobacteriaceae*, which are oxidase-negative, from other gram-negative bacilli.

**PRINCIPLE:**

Cytochromes, found principally in organisms that can utilize free oxygen, are heme-containing proteins involved with oxidative phosphorylation. The enzyme cytochrome oxidase catalyzes the oxidation of cytochrome C, transferring the electrons to the terminal electron acceptor, oxygen. The oxidase reagent tetramethyl-p-phenylenediamine reacts with the oxidized cytochrome C producing a dark purple color.

**FORMULA:**

Approximate.

- (1) **Oxidase Reagent (powder):**  
*N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride ..... 50.0 mg/tube*
- (2) **Oxidase Reagent (liquid)**  
Same as (1) above except the powder is suspended in Dimethyl Sulfoxide.

**PRECAUTIONS:**

For in vitro diagnostic use. Observe all safety precautions consistent with the hazard(s) stated on the product label and/or Material Safety Data Sheet. This reagent may be carcinogenic. Do not ingest.

**Storage:** Upon receipt, store at the recommended temperature away from light. Avoid exposure to extreme heat and cold. Reagent should not be used if there are signs of deterioration or if the expiration date has passed.

**Limitations:** Organisms must be tested with a platinum loop. Iron-containing loops may cause false-positive reactions.

Certain organisms may show a slow or slight positive reaction after the initial 10 seconds has passed. Such results are not considered definitive. A delayed reaction should be retested with a young culture taken from a nonacidic medium. The acid produced in a medium containing a utilizable carbohydrate may cause such a delayed reaction.

The solution prepared with the dry reagent will darken with age, but the dark purple color formed will not interfere with the test reactions. This solution loses sensitivity beyond one week after hydration, and so should be discarded.

**PROCEDURE:\***

**Specimen Collection:** Not applicable since this reagent is used in characterizing isolated organisms from primary isolation. Information on specimen collection may be found in standard reference texts.<sup>2</sup>

**Method of Use:** To prepare the stock solution, pipette 5 ml of deionized water into a tube containing the oxidase reagent.



To test an organism, moisten a piece of filter paper with the Oxidase Reagent. Remove a portion of a colony with a sterile platinum loop and smear on the moistened filter paper. A dark purple color will appear if the organism possesses the enzyme.

**Interpretation:**

Positive: A dark purple color appears on the filter paper within 10 seconds at the point of inoculation.

Negative: No color development within 10 seconds on the filter paper where the organism is deposited. See "Limitations."

**Materials Required but Not Provided:** Standard microbiological supplies and equipment such as loops, needles, pipettes, and filter paper are not provided.

**QUALITY CONTROL:\***

**Microorganisms Used (ATCC #):**

*Pseudomonas aeruginosa* (27853)

*Escherichia coli* (25922)

**Expected Results:**

(+)

(-)

Key: See "Interpretation"

**User Quality Control:** Check for signs of deterioration. Test the efficacy of the reagent daily using the quality control organisms described above.

**BIBLIOGRAPHY:**

1. Blazevic, D., and C. Ederer, *Principles of Biochemical Tests in Diagnostic Microbiology*, John Wiley and Sons, New York, 1975.
2. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.

\*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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