

**INTENDED USE**

TestOxidase™ Reagent is a qualitative method for the determination of bacterial cytochrome oxidase.

**SUMMARY AND EXPLANATION**

Cytochromes are heme-containing proteins and are oxidative enzymes in the respiratory chain of bacteria which utilize free oxygen as a terminal electron acceptor. TestOxidase™ Reagent reacts with oxidized cytochrome C to form a coloured complex. Therefore, the test is positive for bacteria, which contain cytochrome C as part of their respiratory chain, and negative for bacteria, which do not contain cytochrome C.

**PRINCIPLE OF THE PROCEDURE**

TestOxidase™ Reagent is based upon the oxidation of tetramethyl-*p*-phenylenediamine by bacterial cytochrome in the presence of atmospheric oxygen to form a purple coloured compound (Wurster's blue).

**REAGENTS**

Pro-Lab TestOxidase™ Reagent PL.390 is supplied as 15 ml stable liquid reagent in an opaque dropper vial. Each vial contains sufficient tetramethyl-*p*-phenylenediamine solution for 400 tests.

**FORMULA**

0.3% N,N,N',N'- tetramethyl-*p*-phenylenediamine in 0.003 M buffer containing reducing agents and organic stabilizers.

**PRECAUTIONS**

1. Pro-Lab TestOxidase™ Reagent PL.390 is intended for *in vitro* use only.
2. Do not use the TestOxidase™ Reagent after the expiry date shown on the product label.
3. Avoid contact with skin, eyes and clothing. Flammable.
4. The reagent should appear colourless, cloudy or very light tan. Do not use if the TestOxidase™ Reagent is purple.
5. During and after use, handle all materials in a manner conforming to Good Laboratory Practices and consider at all times that material under test should be regarded as a potential biohazard if mishandled.
6. The procedures, storage conditions, precautions and limitations specified in these directions must be adhered to in order to obtain valid information.

**STORAGE**

Store Pro-Lab TestOxidase™ Reagent PL.390 at controlled room temperature (15–30°C) in the original container. Do not freeze or over-heat. Protect from light. Keep the screw cap tightly closed. Product stored under these conditions will be stable until the expiry date shown on the label.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Inoculating loop
2. Filter paper strips or pads
3. Incubator
4. Supplemental media
5. Quality control organisms
6. Cotton swabs

**PROCEDURE**

Clinical specimens should be inoculated onto appropriate isolation media to obtain well-defined isolated colonies for testing. Only fresh isolates (18–24 hours old) should be used since older cultures or deteriorated media may lead to aberrant results.

**1. Direct (Colony) Method:**

- Add one drop of TestOxidase™ Reagent to a well-isolated colony on the surface of recommended agar medium.
- Observe the colony for a colour change within 30 seconds. (If the test isolate produces excessively mucoid or slimy colonies, allow up to 1 minute for colour development.)

**2. Filter Paper Method:**

- Add 1 to 2 drops of TestOxidase™ Reagent to any convenient size of filter paper. Wait 1 to 2 minutes for proper reagent redistribution.
- Using a wooden mixing stick or disposable inoculating loop (nichrome wire loops are not recommended), remove a medium size colony from the surface of the recommended agar medium and rub the inoculum onto the reagent-saturated area of the filter paper.
- Observe the filter paper for colour change within 30 seconds.

**3. Swab Method:**

- Using a cotton swab, remove a medium sized colony from the surface of the agar medium.
- Add 1 to 2 drops of TestOxidase™ Reagent on to the culture on the swab.
- Observe the swab for a colour change within 30 seconds.

**QUALITY CONTROL**

For laboratory quality control, the following reference strains are recommended:

Organism	Expected Results
<i>Escherichia coli</i> ATCC 25922/NCTC 12241	Negative
<i>Pseudomonas aeruginosa</i> ATCC 27853/NCTC 12934	Positive

Alternate / Additional Reference Strain:

<i>Pseudomonas aeruginosa</i> ATCC 25668/NCTC 10662	Positive
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Each lot of TestOxidase™ Reagent is subject to quality control at Pro-Lab.

**INTERPRETATION OF RESULTS**









Negative reaction: The absence of a distinct blue or purple colour.  
 Positive reaction: The production of a distinct blue or purple colour.





**LIMITATIONS OF THE PROCEDURE**

1. Pro-Lab TestOxidase™ Reagent PL.390 is for *in vitro* diagnostic use only, and should be used by properly trained individuals.
2. Results inconsistent with other biochemical reactions or with the organism should be repeated.
3. If conflicting information is obtained, the user should conduct additional testing procedures to resolve difficulties.

**REFERENCES**

1. Kovacs, N. 1956. Nature 178:703.
2. Cowan, S.T. and K.J. Steel. 1966. Manual for the Identification of Medical Bacteria, Cambridge University Press, pp.22 & 148-149.
3. Steel, K.J. 1962. J. Appl. Bacteriol. 25:445.
4. Steel, K.J. 1961. J. Gen. Microbiol. 25:297.
5. Lennette, E.H., A. Balows, W.J. Hausler Jr. and H.J. Shadomy. 1985. Manual of Clinical Microbiology, 4th Edition.

	= Use by
	= Lot number
	= Catalogue number
	= Manufacturer
	= Authorized Representative in the European Community
	= In vitro diagnostic medical device
	= Temperature limitation
	= Consult instructions for use

<p><b>EU</b></p>	  <p><b>Danger</b> Highly flammable liquid and vapour. Causes serious eye irritation. May cause drowsiness or dizziness.</p> <p>Wear protective gloves. Wear protective clothing. Wear eye or face protection. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. IF INHALED: Remove person to fresh air and keep comfortable for breathing. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. Store locked up. Dispose of contents and container in accordance with all local, regional, national and international regulations.</p> <p>Contains Tin dichloride. May produce an allergic reaction.</p> <p><b>Hazardous ingredients: Isopropyl alcohol</b></p>
<p><b>United States / Canada</b></p>	  <p><b>Danger</b> Highly flammable liquid and vapor. Causes serious eye irritation. May cause an allergic skin reaction. May cause drowsiness or dizziness.</p> <p>Wear protective gloves. Wear eye or face protection. Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Use explosion-proof electrical, ventilating, lighting and all material-handling equipment. Use only non-sparking tools. Take precautionary measures against static discharge. Keep container tightly closed. Use only outdoors or in a well-ventilated area. Avoid breathing vapor. Wash hands thoroughly after handling. Contaminated work clothing must not be allowed out of the workplace. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or physician if you feel unwell. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water or shower. IF ON SKIN: Wash with plenty of soap and water. Wash contaminated clothing before reuse. If skin irritation or rash occurs: Get medical attention. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical attention. Store locked up. Store in a well-ventilated place. Keep cool. Dispose of contents and container in accordance with all local, regional, national and international regulations.</p>