OXIDASE REAGENT

INTENDED USE

Oxidase reagent is a liquid ready-to-use reagent that is used in procedures to detect cytochrome oxidase activity in bacteria.

SUMMARY AND EXPLANATION

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 oxidasepositive 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.

PRINCIPLE

The oxidase test is based on bacterial production of an intracellular oxidase enzyme and some organisms may produce more than one type of oxidase enzyme. These enzymes participate in the cellular respiration process and catalyze removal of hydrogen from a substrate using oxygen as a hydrogen acceptor. The active substrate in oxidase reagent, N,N,N,Ntetramethyl-p-phenylenediamine dihydrochloride, acts as an artificial electron acceptor for the enzyme oxidase and is oxidized to form the colored compound Wurster's blue. Wurster's blue is a purple compound that is readily visible and signifies a positive reaction.

REAGENTS (FORMULA)

N,N,N',N'-tetramethyl-p-phenylenediamine Dihydrochloride	12.0	g/l
Stabilizing Agent	0.2	g/l

PROCEDURE

Direct Plate Procedure

1. Allow reagent adequate time to reach room temperature prior to use.

2. Add 1-2 drops of Oxidase Reagent directly to afew suspected colonies from a culture plate grown on an appropriate medium such as blood agar or chocolate agar. (Do not flood the entire plate with reagent)

3. Observe for a purple color change within 30 sec.

Indirect Paper Strip Procedure

1. Allow reagent adequate time to reach room temperature prior to use.
2. Place a small piece of filter paper into a sterile petri dish.
3. Moisten the filter paper with 1 to 2 drops of Oxidase Reagent.
4. Touch the colony to be tested with the end of a sterile wooden applicator or platinum loop.
5. Smear the colony onto the filter paper.
6. Observe for a purple color change within 30 sec.

Swab Procedure

1. Allow reagent adequate time to reach room temperature prior to use.
2. Saturate a piece of filter paper with Oxidase reagent.
3. Using a sterile swab, pick a colony of interest and touch it lightly.
4. Rub the swab onto the filter paper.
5. Observe for a purple color change within 30 sec on the swab, **not the filter paper**.

EXPECTED RESULTS

For all procedures, a positive reaction is a purple color change occurring within 30 seconds. Oxidase positive colonies typically take 10 seconds to produce a positive color reaction; reactions occurring between 30 and 60 seconds should be classified as a delayed positive and retested. For the direct plate procedure, oxidase-positive colonies will adopt a purplish-black coloration and the reagent may also impart color to the surrounding medium.

A negative result is no color change after 1 minute or a color change that occurs after 1 minute.

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.

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.

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.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 2°C for 18-24 hours	Oxidase-negative; no color development within 10-20 seconds
Pseudomonas aeruginosa ATCC 27853	35 ± 2°C for 18-24 hours	Oxidase-positive; blue/purple color develops within 10-20 seconds

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WARNING AND PRECAUTIONS

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

STORAGE AND SHELF LIFE

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

DISPOSAL

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

BIBLIOGRAPHY

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