OXIDASE REAGENT

- For in vitro use only - Catalogue No. RO95

Our Oxidase Reagent is used to detect the presence of oxidase enzymes produced by a variety of bacteria.

Detection of these enzymes allows for differentiation and presumptive identification of some bacterial species when used in conjunction with other biochemical tests. The oxidase test can be used to differentiate between genera:

1. *Moraxella* (+) and *Neisseria* (+) from *Acinetobacter* (-)
2. *Aeromonas* (+), *Plesiomonas shigelloides* (+), and *Vibrio* (V+) from other *Enterobacteriaceae* (-)

Aid in species differentiation of nonenteric gram-negative organisms:

1. *Bacteroides distasonis* (+), *B. eggerthii* (+), and *B. ureolyticus* (+) from other *Bacteroides* spp. (-)
2. *Bartonella felis* (+), *B. quintana* (w+), and *B. vinsonii* (w+) from *B. bacilliformis* (-), *B. elizabethae* (-), and *B. henselae* (-)
3. *Bordetella parapertussis* (+) from other *Bordetella* spp. (-)
4. *Burkholderia gladioli* (-) and *B. mallei* (V) from *B. cepacia* (+) and *B. pseudomallei* (+)
5. *Capnocytophaga canimorsus* (w+) and *C. cynodegmi* (+) from *C. gingivalis* (-), *C. ochracea* (-), and *C. sputigena* (-)
6. *Haemophilus aphrophilus* (-), *H. parasuis* (-), *H. paragallinarum* (-), and *H. segnis* (-) from other *Haemophilus* spp. (+)
7. *Pseudomonas syringae* (-), *P. viridiflava* (-) from other *Pseudomonas* spp. (+)
8. *Vibrio gazogenes* (-) and *V. metschnikovii* (-) from other frequently isolated *Vibrio* spp. (+)

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,N-tetramethyl-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.

Our formulation was devised by Kovacs and is often referred to as Kovacs Oxidase Reagent. The addition of a stabilizing agent minimizes auto-oxidation of the reagent and provides an extended shelf life to the product.

Formulation per 100 mL

N,N,N,N-Tetramethyl-p-phenylenediamine Dihydrochloride	0.60 g
Stabilizing Agent	0.02 g
Dimethyl Sulfoxide (DMSO)	100.0 mL

Recommended 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 a few 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**.

Interpretation of 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.

- *The reagent is a clear, colorless liquid, if it becomes discoloured or cloudy discard the reagent*
- *Do not attempt to perform the oxidase test on colonies grown on medium containing glucose as fermentation inhibits oxidase activity and may result in false negatives*
- *Do not use loops that contain iron or nichrome since false-positive reactions may result from surface oxidation products formed during flame sterilization*
- *Using the oxidase test to aid in identification of Neisseria requires doing a gram stain on all oxidase-positive colonies to confirm that they are diplococci*
- *Oxidase reagent can auto-oxidize over time therefore regular quality control checks should be performed on known positives and negatives to ensure adequate reagent performance*

Quality Control

Organism	**Expected Results**	
Pseudomonas aeruginosa ATCC 27853	+ve	Dark purple color
Escherichia coli ATCC 25922	-ve	No color change

Storage and Shelf Life

Our Oxidase Reagent should be stored at room temperature, away from light. Under these conditions, it has a shelf life of 16 weeks from the date of manufacture.

References

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6. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St Louis: Mosby, 1998.

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