

Oxidase Reagent (Gordon McLeod Reagent)

Intended use

An oxidase reagent is used to detect the presence of an enzyme cytochrome oxidase found in some bacteria.

Summary

Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen, the final electron acceptor in some electron transport chains. Presence of cytochrome oxidase can be detected through the use of an oxidase reagent (Gordon-McLeod Reagent), which acts as an electron donor to cytochrome oxidase. If the bacteria oxidize the reagent, it turns dark purple due to the formation of indophenol blue indicating a positive reaction. This test is used for screening species of *Neisseria*, *Aeromonas*, *Vibrio*, *Campylobacter* and *Pseudomonas*, which show a positive reaction, and for *Enterobacteriaceae*, which show a negative reaction.

Principle

The Gordon-McLeod Reagent serves as an alternate substrate for the cytochrome oxidase reaction. In the reduced state N, N, N', N-tetramethyl-p-phenylenediamine dihydrochloride is colourless but when oxidized it becomes dark purple due to the formation of indophenol blue indicating a positive reaction.

Reagents/contents

The Microxpress® Gordon-McLeod Reagent is a reagent set for laboratory use only.

The Gordon-McLeod Reagent comprises of:

1. 0.5% N, N, N', N-tetramethyl-p-phenylenediamine dihydrochloride.
2. Distilled water

Storage and stability

1. Store the Gordon-McLeod Reagent at 2°C-8°C away from light.
2. Stability of the Gordon-McLeod Reagent is as per the expiry date mentioned on the label.

Procedure

Preparation of Inoculum

1. Isolate the organism to be identified on Nutrient Agar or Brain Heart Infusion Agar.
2. Pick up a single isolated colony and inoculate it in 4-5 mL Brain heart Infusion Broth.
3. Incubate at 37°C for 6-8 hours until inoculum turbidity is between 0.1-0.2 at 620 nm. Alternatively, a homogenous suspension made in 2-3 mL sterile saline adjusted to a turbidity of 0.1-0.2 at 620 nm can also be used as inoculum.

Test procedure

Method 1

1. Inoculate an aliquot (1 mL) of a suitable medium Brain Heart Infusion Broth with the above-prepared inoculum (approx. 100 mL) and incubate for 6-8 hours at 35°C-37°C.
2. Observe for growth.
3. Add 1-2 drops of Gordon-McLeod Reagent.
4. Observe for colour change.

OR

Method 2

1. Plate out a pure culture on a suitable medium like a Brain Heart Infusion Agar.
2. Incubate at 35°C-37°C for 18 - 24 hours.
3. Add 3 - 5 drops of Gordon-McLeod Reagent on a filter paper placed in a petriplates.
4. With a sterile platinum loop (not nichrome), plastic loop or glass rod, smear some of the colonies on the prepared filter paper.
5. Observe for colour change.

Warning

Gordon-McLeod Reagent is irritating to the eyes, respiratory system and skin. Do not breathe dust and wear suitable protective clothing.

Appearance: Amber coloured with an alcoholic odour.

Interpretation of results

1. Formation of purple color within 60 seconds indicates a positive test.
2. No change in color or delayed colour indicates a negative test.

Quality control

Organisms (ATCC)

Pseudomonas aeruginosa Strain Boston 41501 (27853)
Escherichia coli (25922)

Observed reaction in Gordon McLeod test

+

-

Key:

+ = Formation of purple colour in 60 seconds
- = No change in colour or delayed colour

Precautions / Limitations

1. The Gordon-McLeod Reagent is an *in-vitro* diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The Gordon-McLeod Reagent cannot be used directly on clinical specimens. Only pure cultures should be used to obtain optimum results.
3. At times, the organism may give contradictory results because of mutation or media used for isolation, cultivation and maintenance. Results are prominent when fresh and enriched culture is used.
4. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
5. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of gram reaction, morphology, and other biochemical and serological tests.
6. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.
7. Do not keep the bottle open for long time. Avoid exposure to light.
8. A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds. A 'delayed' positive reaction is indicated by colouration in 10-60 seconds and a negative reaction is indicated by the absence of colouration or any colouration that shows later than 60 seconds.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

1. Practical Medical Microbiology, Mackie & McCartney, 13th edition 1989, Edited by J. G. Collee, J. P. Duguid.
2. Diagnostic Microbiology, Bailey & Scott, 9th Edition, Mosby 1994.
3. Clarke P.H. and S.T. Cowan, Biochemical Methods For Bacteriology, J. Gen. Microbiol., 1952, Vol. 6: 187-197.
4. The Taxonomic Significance Of Fermentative Versus Oxidative Metabolism Of Carbohydrates By Various Gram-Negative Bacteria, By Rudolph Hugh And Einar Leifson.
5. Practical Laboratory Test For The Identification Of *Pseudomonas Aeruginosa*, By W. L. Gaby And C. Hadley.
6. Data on file: Microexpress, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.

204070150100

Product

Oxidase Reagent (Gordon McLeod Reagent)

Pack Size

100 mL

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
