TDA Reagent

Intended Use
For phenylalanine deamination reaction in differentiating *Proteus* from other members of *Enterobacteriaceae*.

Summary
It is a widely known fact that *Proteus, Providencia* and *Morganella* species have the ability to deaminate phenylalanine to phenylpyruvic acid by the enzymatic activity. TDA reagent (Ferric Chloride reagent) detects the presence of this phenylpyruvic acid. This reagent is useful in differentiating *Proteus* from other members of the *Enterobacteriaceae* by the ability of organisms in the genera within the *Proteeae* to deaminate phenylalanine to phenyl pyruvic acid by the enzymatic activity.

Principle
This test detects the ability of an organism to oxidatively deaminate phenylalanine with production of phenylpyruvic acid. On addition of TDA reagent to the medium, after incubation, phenyl pyruvic acid produced reacts with ferric salts in the reagent to give a green colour appearance indicating a positive reaction. No change in colour indicates negative reaction.

Reagents/contents
The Microxpress® TDA Reagent (Ferric Chloride Reagent) is a reagent set for laboratory use only.
The TDA Reagent (Ferric Chloride Reagent) comprises of:

1. 10% Ferric Chloride.

Storage and stability
1. Store the TDA Reagent (Ferric Chloride Reagent) at 15°C-25°C away from light.
2. Stability of the TDA Reagent (Ferric Chloride 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 containing the substrate phenylalanine with the above-prepared inoculum (approx. 100 µL) and incubate for 4 hours at 35°C-37° C.
2. Observe for growth.
3. Add 2-3 drops of TDA Reagent (Ferric Chloride Reagent).
4. Observe for appearance of green colour. The appearance of colour may take around 5 minutes.

OR

Method2

1. Plate out a pure culture on a suitable medium like a Phenylalanine Agar slant.
2. Incubate at 35°C-37° C for 18-24 hours. If the inoculum is sufficiently heavy, a 4-hour incubation period is sufficient.
3. Add 3-5 drops of TDA Reagent (Ferric Chloride Reagent) down the agar slope.
4. Observe for appearance of green colour.

Appearance: Light yellow coloured clear solution.

Interpretation of results

1. Formation of deep green colour in 5 minutes indicates a positive test.
2. No colour change indicates a negative test.

Quality control

Organisms (ATCC)	Observed result
Proteus hauseri (13315)	Appearance of green colour in 5 minutes
Klebsiella aerogenes (13048)	No colour change

Precautions/limitations

1. The TDA Reagent (Ferric Chloride Reagent) is an in vitro diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The TDA Reagent (Ferric Chloride 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. Interpret the results within five minutes after the addition of the TDA Reagent (Ferric Chloride Reagent) as the green colour formed fades quickly.
5. 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.
6. 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.
7. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.

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, 13'" edition 1989, Edited by J. G. Callee, J.P. Duguid.
2. Diagnostic Microbiology, Bailey & Scott, 9'" Edition, Mosby1994.
3. Clarke P.H. And S.T. Cowan, Biochemical Methods For Bacteriology, J. Gen. Microbial., 1952, Vol. 6: 187-197.
4. Combined medium to determine deoxyribonuclease activity and phenylalanine deamination by Enterobacteriaceae, Thomas R. Oberhofer and Louise Maddox, Applied Microbiology, Feb. 1970, Vol. 19, No. 2 p: 385-386.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Pack Size
204200580010	TDA Reagent	10 mL