



## FERRIC CHLORIDE REAGENT

- For in vitro use only -

Catalogue No. RF40

Our Ferric Chloride Reagent is used to detect the presence of the enzyme phenylalanine deaminase produced by some bacteria.

Organisms possessing the enzyme, phenylalanine deaminase, can convert phenylalanine to the  $\alpha$ -keto acid phenylpyruvic acid. This  $\alpha$ -keto acid can react with ferric chloride reagent to form a green cyclic compound; exposing the culture tube to oxygen increases the rate of production and the strength of this reaction.

The phenylalanine deaminase test is used primarily to differentiate among the members of the family *Enterobacteriaceae*. Within this family only *Morganella* spp., *Proteus* spp., *Providencia* spp., *Pantoea agglomerans* (20% of strains), *Enterobacter sakazakii* (50% of strains), *Rahnella aquatilis*, and *Tatumella ptyseos* can deaminate phenylalanine and produce a positive reaction. The phenylalanine deaminase test can also aid in species differentiation: *Afipia felis* (+) from *A. broomeae* (-) and *A. clevelandensis* (-).

Our Ferric Chloride Reagent can be used with two different mediums. The most commonly used medium for performing the phenylalanine deaminase test is Phenylalanine Agar (Dalynn TP59), although a rapid buffered phenylalanine solution has also been described.

### Formulation per 100 mL

Ferric Chloride ..... 10.0 g  
Hydrochloric Acid (2%) ..... 100.0 mL

### Recommended Procedure (Conventional)

- Using a sterile inoculating loop, touch several similar colonies obtained from an overnight culture and streak the surface of the Phenylalanine Agar Slant (Dalynn TB59).

- Incubate at 35°C for 24 hours.
- Add four to five drops (0.5-mL) of Ferric Chloride Reagent and rotate the tube gently.
- Check for a color reaction immediately. All positive color reactions must be read within five minutes.

### Interpretation of Results

Positive: Development of a green color within 5 minutes

Negative: No color change (yellow)

- Positive color reactions will disappear ten minutes after the addition of Ferric Chloride Reagent therefore ensure results are read within a 5 minute interval
- A few non-fermenting gram-negative bacilli are also phenylalanine deaminase positive and can produce a positive reaction

### Quality Control

Organism	Expected Results	
<i>Proteus mirabilis</i> ATCC 12453	+ve	Green color change
<i>Escherichia coli</i> ATCC 25922	-ve	No color change

### Storage and Shelf Life

Our Ferric Chloride Reagent should be stored at 4°C to 8°C and protected from light. Under these conditions, the reagent has a shelf life of 52 weeks from the date of manufacture.

## **References**

1. Henriksen SD, Closs K. The production of phenylpyruvic acid by bacteria. *Acta Pathol Microbiol Scand* 1938; 15:101-13.
2. Henriksen SD. A comparison of the phenylalanine acid reaction and the urease test in the differentiation of *Proteus* from other enteric organisms. *J Bacteriol* 1950; 60:225-31.
3. Stewart DJ. The phenylalanine test on Kligler's iron agar for the identification of *Proteus*. *Nature* 1959; 183:1537-8.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol I. Washington, DC: ASM, 1992.
5. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3<sup>rd</sup> ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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