

Instructions for Use

FERRIC CHLORIDE REAGENT, 10%

Cat. no. Z63	Ferric Chloride Reagent, 10%	15ml
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INTENDED USE

Hardy Diagnostics Ferric Chloride Reagent, 10% is recommended for use in determining hippurate hydrolysis, phenylalanine deaminase, and tryptophan deaminase reactions in bacteria.

SUMMARY

The hippurate test has been recommended for use in the presumptive identification and differentiation of group B streptococci from other beta-hemolytic streptococci. In the hippurate test, hippurate is hydrolyzed to glycine and benzoic acid. The addition of acidified ferric chloride combined with benzoic acid form a brown precipitate of ferric benzoate.

Phenylalanine and tryptophan deaminase reactions are used in the identification and differentiation of gram-negative bacilli. Oxidative deamination of these amino acids yields keto acids phenylpyruvate and indolepyruvate respectively, and ammonia. The addition of acidified Ferric Chloride Reagent to the keto acids produces green or brown colored compounds, respectively.

REAGENT FORMULA*

Ferric Chloride	100.0gm
Hydrochloric Acid, 37%	25.0ml
Deionized Water	975.0ml

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Upon receipt store 2-30°C. Products should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Specimen Collection: This product is not intended for primary isolation of patient specimens. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Hippurate Test: Heavily inoculate 5ml of Sodium Hippurate Broth (Cat. no. Z52) with test organism. Concurrent isolation of positive and negative control organisms is also recommended. Incubate 24-48 hours at 35°C. Centrifuge the medium to pack the cells and transfer 0.8ml of the clear supernatant to a small test tube. Add Ferric Chloride Reagent.

Note: It is recommended that the amount of Ferric Chloride Reagent to be used in the assay be titrated to insure optimal performance.⁽¹⁾

Ferric Chloride Reagent Titration: To 0.8ml amounts of uninoculated Hippurate Broth (Cat. no. Z52) control tubes, quickly add 4, 6, 8, and 10 drops of Ferric Chloride Reagent. Mix thoroughly. Let stand for 10 minutes with occasional shaking. The smallest quantity of acidified Ferric Chloride Reagent giving a clear solution is the quantity to be used in the assay. Examine for a precipitate which persists for longer than 10 minutes.

Phenylalanine or Tryptophan Deaminase Test: Heavily inoculate Phenylalanine (Cat. no. L21) or Tryptophan (Tryptone Broth, Cat. no. R40) media with the test organism. Incubate 18-24 hours at 35°C. Add 4 to 5 drops of Ferric Chloride Reagent directly to the culture. Examine for the appearance of a green color when using Phenylalanine media or a brown color when using Tryptophan (Tryptone Broth) media.

If ferric chloride is to be used for a commercial identification test strip, such as API[®], consult the manufacturer's literature.

INTERPRETATION OF RESULTS

A positive hippurate reaction is denoted by the appearance of a brown precipitate that persists for 10 minutes.

Positive phenylalanine and tryptophan deaminase reactions are denoted by the appearance of a green or brown color, respectively, within five minutes. Positive phenylalanine reactions must be interpreted immediately since the green color fades quickly.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Hippurate hydrolysis, phenylalanine deaminase and tryptophan reactions may be used as aids in the identification of beta-hemolytic streptococci and gram-negative bacilli, respectively. Additional testing, using pure culture, is recommended for complete identification.

In the hippurate test, the final concentration of ferric ion is extremely important. It is recommended that titration of the amount of reagent to be used be performed in order to minimize false-positive reactions.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, Hippurate Broth (Cat. no. Z52), Phenylalanine media (Cat. no. L21), Tryptone Broth (Cat. no. R40), incinerators, incubators, pasteur pipets, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Results
<i>Streptococcus agalactiae</i> ATCC® 12386	Hippurate Hydrolysis: Positive Phenylalanine Deaminase: N/A Tryptophan Deaminase: N/A
<i>Streptococcus pyogenes</i> ATCC® 19615	Hippurate Hydrolysis: Negative Phenylalanine Deaminase: N/A Tryptophan Deaminase: N/A
<i>Proteus mirabilis</i> ATCC® 12453	Hippurate Hydrolysis: N/A Phenylalanine Deaminase: Positive Tryptophan Deaminase: Positive
<i>Escherichia coli</i> ATCC® 25922	Hippurate Hydrolysis: N/A Phenylalanine Deaminase: Negative Tryptophan Deaminase: Negative

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Ferric Chloride Reagent, 10% should appear clear, and yellow in color.



Showing positive phenylalanine deaminase reaction.

Ferric Chloride Reagent, 10% (Cat. no. Z63) added to Phenylalanine slant (Cat. no. L21) with heavy growth of *Proteus mirabilis* (ATCC® 12453). *P. mirabilis* was incubated aerobically for 24 hours at 35°C.



Showing negative phenylalanine deaminase reaction.

Ferric Chloride Reagent, 10% (Cat. no. Z63) added to Phenylalanine slant (Cat. no. L21) with heavy growth of *Escherichia coli* (ATCC® 25922). *E. coli* was incubated aerobically for 24 hours at 35°C.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
2. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

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ATCC is a registered trademark of the American Type Culture Collection.

IFU-10424[A]



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[Ordering Information](#)

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