

Instructions for Use

NINHYDRIN REAGENT

Cat. no. Z70	Ninhydrin Reagent	15ml
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INTENDED USE

Hardy Diagnostics Ninhydrin Reagent is recommended for use in rapid determination of sodium hippurate hydrolysis reactions in bacteria.

SUMMARY

The hippurate test is a qualitative procedure for determining the ability of group B streptococci, as well as other bacteria, to hydrolyze sodium hippurate to benzoic acid and glycine. The development of a purple color after the addition of ninhydrin indicates the splitting of hippurate by the detection of glycine.

REAGENT FORMULA

Ingredients per liter:*

Ninhydrin	35.0gm
Acetone	500.0ml
Butanol	500.0ml

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

STORAGE AND SHELF LIFE

Upon receipt store at 2-30°C. Reagent is light sensitive; protect from light. Products should not be used if there are any signs of deterioration or if the expiration date has passed.

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.

Warning: Flammable - Keep away from heat and flames. Use adequate ventilation.

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 organisms.

Method of Use: Place hippurate disk in a tube with 0.5mL sterile water. Heavily inoculate with 18-24 hour culture of the test organism. Concurrent inoculation of positive and negative control organisms is also recommended. Incubate for at least two (2) hours, but not more than 6 hours at 35°C. Add five (5) drops of Ninhydrin Reagent, mix well. Reincubate tubes 30 minutes and read reaction.

INTERPRETATION OF RESULTS

A positive hippurate reaction is denoted by the appearance of a deep purple-blue color. A negative hippurate reaction is indicated by no color change.

Note: Do not incubate longer than 30 minutes because a false-positive reading could result.

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.

Rapid sodium hippurate hydrolysis reactions may be used in the presumptive identification of group B beta-hemolytic streptococci. Additional testing, using pure culture, is recommended for complete identification.

Some strains of group D streptococci may give a weak positive result. Group D can be differentiated from group B by esculin hydrolysis. Only group D streptococci hydrolyze esculin yielding a blackening of the medium.

Incubation of test culture for more than 30 minutes after the Ninhydrin Reagent is added can yield false-positive results.

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, 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	Reaction
<i>Streptococcus agalactiae</i> ATCC® 12386	Positive; purple color change
<i>Streptococcus pyogenes</i> ATCC® 19615	Negative; no color change

USER QUALITY CONTROL

It is recommended that each new lot of reagent be tested with known positive and negative controls and retested at least each week of use thereafter.⁽³⁾

PHYSICAL APPEARANCE

Ninhydrin Reagent should appear clear, colorless to light yellow.

REFERENCES

1. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
2. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
3. 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.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
6. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.

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

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[Ordering Information](#)

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