IFU

Instructions for Use

RAPID ORNITHINE

Cat. no. K279	Rapid Ornithine, 13x100mm Tube, 1ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Rapid Ornithine can be used to detect ornithine decarboxylase activity in as little as two to four hours. This test will assist in the identification of *Enterobacteriaceae* and *Staphylococcus lugdunensis*.

SUMMARY

Conventional methods for detecting ornithine decarboxylase activity require an extended period of incubation. The tests generally involve the fermentation of glucose, which lowers the pH of the medium to the optimum hydrogen ion concentration for decarboxylase activity. Decarboxylation results in the formation of amines that will raise the pH. Positive reactions are usually obtained after 18 to 24 hours of incubation, but some strains require up to four days incubation. An overlay of mineral oil acts as a barrier to oxygen and prevents alkalinization of the surface of the medium.

Fay and Barry modified the conventional decarboxylase medium by removing the glucose and decreasing the pH to 5.5.[7] A small volume of the broth is heavily inoculated and then overlayed with sterile mineral oil. With the Hardy Diagnostics Rapid Ornithine, results can be obtained after only two to five hours of incubation at 35 to 37ºC.

The test can be used to determine ornithine decarboxylase activity in the family *Enterobacteriaceae* and can be used to differentiate *Staphylococcus lugdunensis* from other species of *Staphylococcus*. It is especially useful to differentiate *Proteus mirabilis* from other *Proteus* spp., which are ornithine decarboxylase negative. Some strains of *Proteus* require five hours of incubation to detect a positive result.

Decarboxylase basal media consists of peptones and yeast extract which supply nitrogenous and other nutrients necessary for bacterial growth. Bromcresol purple is a pH indicator. The amino acid ornithine is added to detect the production of the enzyme ornithine decarboxylase.

FORMULA

Ingredients per liter of deionized water:*

L-Ornithine	10.0gm
Meat Peptone	5.0gm
Yeast Extract	3.0gm
Bromcresol Purple	1.0gm

Final pH 5.6 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2 to 8ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration 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

1. Inoculate a Rapid Ornithine tube by touching the edge of a colony (18-24 hours old) and mix well.

2. Overlay with at least 0.5ml of sterile mineral oil (Cat. no. Z80).

3. Incubate aerobically with loose caps at 35 to 37ºC., for two to four hours. (See Limitations)

INTERPRETATION OF RESULTS

For reference, compare an uninoculated tube with inoculated tubes.

Positive reaction - development of a dark purple to violet color. Must be darker than the uninoculated reference tube.

Negative reaction - no development of color (remains pale yellow to light grayish-purple) or a development of yellow color.

Organisms expected to give a positive result:

- *Edwardsiella* spp.
- *Enterobacter aerogenes*
- *Shigella sonnei*
- *Proteus mirabilis*

- *Staphylococcus lugdunensis*

Organisms expected to give a negative result:

- *Citrobacter freundii*
- *Shigella flexneri*
- *Klebsiella pneumoniae*
- *Proteus hauseri*
- *Staphylococcus epidermidis*
- all other *Staphs* are negative, except some rare strains of *Staphylococcus epidermidis*[5]

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.

Incubating tubes with tight caps may lead to false negative reactions with some *Proteus* spp.

Some strains of *Proteus* require up to five hours of incubation to detect 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, swabs, applicator sticks, sterile mineral oil (Cat. no. Z80), other culture media, incinerators, and incubators, 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	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Staphylococcus lugdunensis ATCC® 49576**	E	2-4hr	35°C	Aerobic	Positive
Proteus mirabilis ATCC® 12453	E	2-5hr	35°C	Aerobic	Positive
Staphylococcus epidermidis ATCC® 12228**	E	2-4hr	35°C	Aerobic	Negative
Proteus vulgaris ATCC® 8427	E	2-5hr	35°C	Aerobic	Negative

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

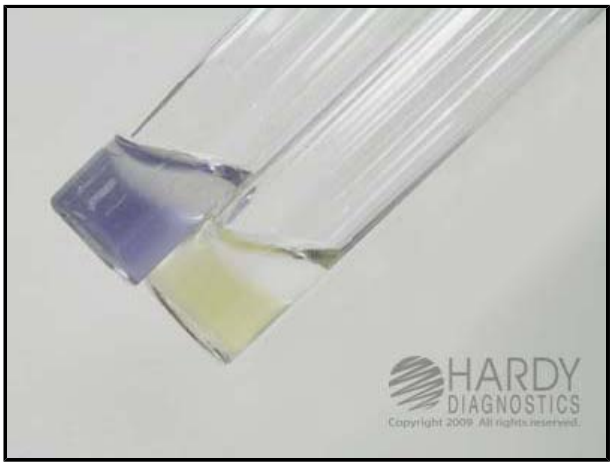
USER QUALITY CONTROL

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

Rapid Ornithine should appear clear and pale yellow to light grayish-purple in color.



LEFT: *Staphylococcus lugdunensis* (ATCC® 49576)
Positive reaction at four hours.
RIGHT: *Staphylococcus epidermidis* (ATCC® 12228)
Negative reaction at four hours.

REFERENCES

1. 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.

2. Jorgensen., et al. *Manual of Clinical Microbiology,* American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

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. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

7. Fay, G.D and Barry, A.L. 1972. Rapid Ornithine Decarboxylase Test for the Identification of *Enterobacteriaceae*. *Applied Microbiology*; Vol. 23, p. 710-713.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]