IFU

Instructions for Use

PHENYLALANINE AGAR

Cat. no. L21	Phenylalanine Agar, 16x100mm Tube, 5.5ml Slant	20 tubes/box

INTENDED USE

Hardy Diagnostics Phenylalanine Agar is recommended for use in the differentiation of gram-negative enteric bacilli based on the ability of the microorganisms to produce phenylpyruvic acid by oxidative deamination.

SUMMARY

In 1950, Hendriksen demonstrated that *Proteus* spp. were able to convert the amino acid phenylalanine to phenylpyruvic acid. Later, Buttiaux et al. developed a culture medium for detecting the formation of phenylpyruvic acid from phenylalanine by members of the *Proteus*, *Providencia*, and *Morganella* groups.[1] This medium was modified by Bynae and further modified by Ewing et al. who simplified Bynae's formula by omitting proteose peptone.[2,3] Hardy Diagnostics Phenylalanine Agar follows the formulation established by Ewing.

The deamination of phenylalanine by oxidative enzymes results in the formation of phenylpyruvic acid. After incubation, an aqueous solution of ferric chloride is added. If phenylpyruvic acid is present, a light to deep green color is produced. Of the *Enterobacteriaceae*, only *Proteus*, *Providencia*, and *Morganella* species possess enzymes capable of deaminating phenylalanine.[4]

FORMULA

Ingredients per liter of deionized water:*

Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Phenylalanine	2.0gm
Dipotassium Phosphate	1.0gm
Agar	12.0gm

Final pH of 7.3 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 15-30ºC. Media should not be used if there are any signs of contamination, deterioration 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 medium is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organisms.

Method of Use:

1. Prior to inoculation, allow the medium to equilibrate to room temperature.

2. Using a loopful of inoculum from an 18-24 hour pure culture, streak the slant surface using a fishtail motion.

3. Incubate the inoculated slant aerobically at 35ºC. for 18-24 hours.

4. Following incubation, apply 4-5 drops of a 10% Ferric Chloride solution (Cat. no. Z63) directly to the slant.

5. Gently agitate the tube and observe for the development of a green color within 1-5 minutes.

INTERPRETATION OF RESULTS

A positive phenylalanine deamination reaction is indicated by the development of a light to dark green color within 1-5 minutes after applying ferric chloride reagent.

A negative test is indicated by the absence of a green color reaction. Negative results will take on a yellow color due to the color of the ferric chloride.

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.

The green color reaction of a positive test fades rapidly. Test results must be interpreted within 5 minutes following the application of ferric chloride or false-negative results may occur.

Slight agitation of the tube containing ferric chloride will dislodge surface colonies and produce a faster more pronounced color reaction.

Refer to the keyword "Limitations", in the Hardy Diagnostics' software program HUGO™, for more information regarding general limitations on culture media.

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, slides, staining supplies, ferric chloride reagent (Cat. no. Z63), other culture media, microscopes, 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	
Proteus mirabilis ATCC® 12453**	E	18-24hr	35°C	Aerobic	Growth; turns green after the addition of 4-5 drops of ferric chloride with agitation, may take 1-5 minutes
Escherichia coli ATCC® 25922**	E	18-24hr	35°C	Aerobic	Growth; ferric chloride remains yellow

* 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

Phenylalanine Agar should appear slightly opalescent, and light amber 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 35ºC.

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 35ºC.

Uninoculated tube of Phenylalanine Agar (Cat. no. L21).

REFERENCES

1. Buttiaux, et al. 1954. *Ann. Inst. Pasteur*; 87:375-386.

2. Ewing, et al. 1957. *Pub Hlth Lab.*; 15:153.

3. Hendriksen. 1950. *J. Bacteriol.*; 60:225.

4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

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

6. 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.

7. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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

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

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