



Instructions for Use

ANAEROBIC PEA (PHENYLETHYL ALCOHOL) AGAR

Cat. no. A90	Anaerobic PEA, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J122	Bacteroides Bile Esculin Agar / Anaerobic PEA, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Anaerobic PEA Agar is recommended for use as an enriched medium for the cultivation and selective isolation of Gram-positive and negative obligate anaerobic bacteria.

SUMMARY

The basal medium of Anaerobic PEA consists of pancreatic digest of casein and enzymatic digest of soybean meal. Both provide amino acids, carbohydrates, and vitamins. The medium is supplemented with yeast extract, sheep blood, vitamin K, hemin, and L-cystine. Yeast extract enhances growth of fastidious microorganisms; sheep blood serves as an indicator of hemolysis; vitamin K promotes growth enhancement of pigmented *Prevotella* spp.; hemin and L-cystine improve the growth of *Clostridium haemolyticum*, *Fusobacterium necrophorum*, certain strains of *Actinomyces israelii*, *Bacteroides thetaiotaomicron*, and thiol-dependent streptococci.⁽⁶⁾ The medium is made selective with the addition of phenylethyl alcohol which inhibits facultative anaerobic Gram-negative bacilli. Anaerobic PEA supports the growth of most Gram-positive and Gram-negative obligate anaerobic bacteria.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	15.0gm
Papaic Digest of Soybean Meal	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	5.0gm
Phenylethanol	2.5gm
Vitamin K	10.0mg
Hemin	5.0mg
L-Cystine	0.4gm
Sheep Blood	50.0ml
Agar	15.0gm

Final pH 7.3 +/- 0.3 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), hemolysis, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat and freezing.

The expiration dating 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 quality control incubation times.

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 blood precautions. 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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

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

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.^(1,2,4,6) It is not recommended that a swab be used for specimen collection. Swabs are prone to drying and may be easily exposed to ambient air an oxygen, which is toxic to obligate anaerobes. The preferred means of anaerobic specimen collection is aspiration with needle and syringe. The specimen should be transferred to an anaerobic transport system in order to protect it from oxygen exposure. Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold.

Method of Use: Allow the plates to warm to room temperature, and the agar surface to dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. A large inoculum should be used in order to minimize the toxic effects of oxygen.⁽⁸⁾ Streak for isolation with a sterile loop. Quickly incubate plates at 35-37°C. for 18-48 hours in an anaerobic atmosphere. Examine for typical colonial morphology and characteristics.

A non-selective medium should be inoculated in parallel to the selective medium for enhanced recovery of anaerobic microorganisms.

INTERPRETATION OF RESULTS

Consult listed references for the identification of colony morphology and further biochemical tests required for identification.^(1,2,4,6)

LIMITATIONS

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

Recovery of some anaerobic microorganisms may necessitate prereduction of the medium by placing it in an oxygen-free holding jar just prior to inoculation.

Many anaerobes are more sensitive to oxygen during the log phase of growth; therefore, it may be necessary to incubate inoculated media for a full 48 hours prior to examination and exposure of the culture to ambient air.

Some organisms for which the medium is designed to isolate may be inhibited by the selective nature of phenylethyl alcohol.

Phenylethyl alcohol is volatile and will dissipate over time with air exposure, thus reducing the selective nature of this medium.

Large numbers of anaerobic bacteria are normally present in the following body sites: throat, gingiva, sputum, gastric contents, small bowel, feces, rectal swabs, surfaces of decubitus ulcers, encrusted walls of abscesses, mucosal lining, eschar, voided urine, vagina or cervix, skin and adjacent mucous. Specimens for anaerobic culture, therefore, should not be collected from these sites.

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, anaerobic transports ([Cat. no. AG25H](#)), other culture media, microscopes, incinerators, anaerobic holding jars ([Cat. no. 16000](#)), anaerobic incubation systems, gas generators ([Cat. no. AN25US](#)), anaerobi indicators ([Cat. no. BR55](#)), 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Bacteroides fragilis</i> ATCC® 25285	A	18-24hr	35°C	Anaerobic	Growth
<i>Proteus mirabilis</i> ATCC® 12453	B	18-24hr	35°C	Aerobic	Partial inhibition; slight growth without swarming

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

** Atmosphere of incubation is enriched with 5-10% CO₂.

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 certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Anaerobic PEA should appear opaque, and dark red in color.



Bacteroides fragilis (ATCC® 25285) colonies growing on Anaerobic PEA Agar (Cat. no. A90). Incubated anaerobically for 24 hours at 35°C.



Proteus mirabilis (ATCC® 12453) growth inhibited on Anaerobic PEA Agar (Cat. no. A90). Incubated aerobically for 24 hours at 35°C.



Clostridium perfringens (ATCC® 13124) colonies growing on Anaerobic PEA Agar (Cat. no. A90) showing double zones of hemolysis. Incubated aerobically for 48 hours at 35°C.

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. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
3. Ellner, et al. 1966. *Am. Journ. Clin. Path.*; 45:502.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
6. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

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IFU-10037[B]



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