IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

# PLET AGAR

| | | |
|---|---|---|
| Cat. no. G153 | PLET Agar, 15x100mm Plate, 20ml | 10 plates/bag |
| Cat. no. P15 | PLET Agar, 15x60mm Contact Plate, 15ml | 10 plates/bag |

## INTENDED USE

Hardy Diagnostics PLET Agar (Polymyxin-Lysozyme-EDTA-Thallous acetate) is used for the detection and isolation of *Bacillus anthracis* from environmental samples, animal products, carcasses, and clinical samples from non-sterile sites. PLET Agar can be useful as a component of a screening program for anthrax.

## SUMMARY

*Bacillus anthracis* is the causative agent of anthrax. Humans become infected when they are inoculated with the spores either by inhalation during exposure to contaminated animal products, or by traumatic introduction.[3] Other *Bacillus* species, such as *B. cereus*, can cause serious infections, but the relative virulence of this and other *Bacillus* spp. is trivial compared with that of *B. anthracis*.

PLET (polymyxin-lysozyme-EDTA-thallous acetate) is a selective media used for the isolation of *Bacillus anthracis* from contaminated specimens. PLET Agar inhibits most contaminating organisms and spore-formers closely related to *B. anthracis*, such as *B. cereus*. The specimens are first heat- or alcohol-shocked, then dilutions of these preparations are spread across the PLET plates.[2] Circular creamy-white to gray-white colonies with a ground-glass texture are subcultured onto appropriate media to test for gamma phage, penicillin susceptibility, lack of motility, and lack of beta-hemolysis for complete identification of *B. anthracis*.[2]

Organic nitrogen, carbon, sulfur, vitamins and trace substances are provided by pancreatic digest of casein, peptic digest of animal tissue, yeast extract, and beef heart infusion. Sodium chloride is added to maintain the osmotic equilibrium. Polymyxin inhibits gram-negative organisms. The combination of EDTA and thallous acetate results in the unique action whereby *B. anthracis* strains are easily recovered, while *B. cereus* strains are generally inhibited. The specific interactions that may be involved are not known at this time.[4]

## FORMULA

Ingredients per liter of deionized water:*

| | |
|---|---|
| Pancreatic Digest of Casein | 9.0gm |
| Peptic Digest of Animal Tissue | 5.0gm |
| Sodium Chloride | 5.0gm |
| Yeast Extract | 4.0gm |
| Beef Heart Infusion | 2.0gm |

| | |
|---|---|
| Sodium Carbonate | 0.325gm |
| EDTA | 300.0mg |
| Thallous Acetate | 40.0mg |
| Lysozyme | 300,000U |
| Polymyxin | 30,000U |
| Agar | 15.0gm |

Final pH 7.4 +/- 0.3 at 25ºC.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or 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 applies to the product in its intact packaging when stored as directed.

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

**Product no. G153:**

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.

**Product no. P15:**

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 laboratory 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

**I. For environmental samples or samples submitted from non-sterile sites:**[2]

1. Solid samples should first be emulsified in sterile deionized water or saline (1:1 wt/vol).

2. Heat-shock the spores and effectively destroy vegetative cells of contaminating organisms by heating at 65ºC. for 15-30 minutes in a waterbath or heatblock (*see note below). Alternatively, alcohol-shock by adding filter-sterilized 95-100% ethanol to the specimen to a final concentration of 1:1 (wt/vol) and hold for 30-60 minutes at room temperature.

3. Using a sterile, plastic spreader (Cat. no. SPRLS01), spread 0.25ml aliquots of undiluted and 1:10, 1:100, and 1:1000 dilutions of a heat- or alcohol-treated suspension of the specimen across PLET, Blood Agar 5% (Cat. no. A10), and Nutrient Agar plates (Cat. no. W10).

4. Incubate at 35-37ºC.

5. Inspect plates for growth after 8 hours and up to 48 hours of incubation.

*** Note:** Treat at 67ºC. for 45-50 minutes if the solution is contained in a polypropylene tube and heat-shocked in a heatblock.

**II. For clinical specimens from normally sterile sites:**

Clinical samples from normally sterile sites should be cultured directly on to Blood Agar 5% (Cat. no. A10) or Nutrient Agar (Cat. no. W51). Do not use the heat- or alcohol-shock procedures on samples from normally sterile sites (e.g. blood, CSF) since *B. anthracis* does not usually form spores while inside the body. *B. anthracis* is easily recoverable on these media after overnight incubation.[2]

**III. For contact plate method of use:**

Allow plates to warm to room temperature, and agar surface to dry. Select a surface to test. Sample the surface by firmly pressing the agar against the test area, using the thumb and second finger to hold the plate, and the first finger to press firmly and evenly on the base. The same amount of pressure should be used for each sample. Do not move the plate laterally, as this spreads contaminants across the agar surface. A rolling motion may be used when slightly curved surfaces are sampled. Areas to be assayed may be divided into grids or sections, and days, as required. Incubate and observe for growth per above instructions.

## INTERPRETATION OF RESULTS

*Bacillus anthracis* will appear as circular creamy-white to gray-white colonies, 1 to 3mm in diameter, with a ground-glass texture. Colonies with this appearance are then subcultured on Blood Agar plates to test for gamma phage, penicillin susceptibility, motility and hemolysis.

Colonies of *B. anthracis* may grow more slowly and will appear smaller and smoother on PLET Agar than on non-

selective media, such as Blood Agar 5% and Nutrient Agar.

Consult the listed references for further procedures for identification of isolates.[1-4]

## 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.

Some strains of spore-forming *Bacillus*, other than *B. anthracis*, may be resistant to the inhibitory agents in PLET Agar. Most gram-negative bacilli will be inhibited on PLET Agar, although some exceptions may occur, such as *Proteus vulgaris*. Staphylococci and enterococci are not inhibited on PLET Agar. A thorough characterization and identification of each isolate is required.

A level C Public Health Laboratory must be informed of any isolates presumptively identified as *Bacillus anthracis*.

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, spreaders, other culture media, swabs, applicator sticks, 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 | |
| *Bacillus thuringiensis* ATCC® 33679 | A | 12-48hr | 35°C | Aerobic | Growth |
| *Bacillus cereus* ATCC® 13061 | A | 24-48hr | 35°C | Aerobic | Growth |
| *Bacillus megaterium* ATCC® 14581 | B | 24-48hr | 35°C | Aerobic | Partial to complete inhibition |
| *Escherichia coli* ATCC® 25922 | B | 12-48hr | 35°C | Aerobic | Partial to complete inhibition |
| *Pseudomonas aeruginosa* ATCC® 27853 | B | 12-48hr | 35°C | Aerobic | Partial to complete inhibition |

* 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

PLET Agar should appear clear, and light to medium amber in color.

## 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. Kinsely, R.F. Selective Medium for *Bacillus anthracis*. *J. of Bacteriology*. Sept., 1966.

5. Turnbull, P.C.B., Boehm R., Cosivi O., Doganay M., Hughs-Jones M. E., Laitha M.K. and DeVos, V. 1998. *Guidelines for the Surveillance and Control of Anthrax in Humans and Animals*. WHO/EMC/ZDI/98.6. World Health Organization, Geneva, Switzerland.




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



HDQA 2207F [D]