# PRODUCT SPECIFICATION SHEET

## PYR Broth (DM1829)

**Intended Use**
PYR Broth (DM1829) is recommended for cultivation of Streptococcus pyogenes.

**Product Summary and Explanation**
*Bacillus anthracis* is a gram positive, endospore-forming, rod-shaped bacterium and is a causative agent of an infectious disease Anthrax. In human anthrax, the bacillus is usually demonstrable in material from a malignant pustule, sometimes in sputum from pulmonary anthrax and also in the blood in the septicemic stage of all forms of the infections. Humans are relatively resistant to anthrax and laboratory workers are rarely infected. However great care should be taken to avoid escape of the long surviving spores into laboratory environment and all the procedures should be carried out in safety cabinet. Although, anthrax cannot spread directly from human to human but anthrax spores can be transported by human clothings, shoes etc. Anthrax in humans is caused by exposure to dead infected animals, consumptions of infected animal tissue or exposure to light density anthrax spores from animal wool, fur, hide, etc. Knisley[1] originally formulated PLET Agar Base which is an excellent selective medium for cultivation of *B. anthracis* [2,3,4] from suspected environmental specimens, animal products or clinical specimens, inhibiting *Bacillus cereus*.

**Principles of the Procedure**
PYR hydrolysis is a presumptive test for both group A and group D enterococcal streptococci (1). The PYR test determines the activity of enzyme L-pyrrolidonyl arylamidase (PYR) produced by Streptococcus pyogenes but not by other bhaemolytic streptococci (2). Free b-napthylamide is then detected by addition of the diazo dye complex, N ,Ndimethylaminocinnamaldehyde. Development of a red colour is indicative of PYR hydrolysis (3). PYR test is a highly sensitive test, which replaces bacitracin and salt tolerance (growth in 6.5% NaCl) tests (1). PYR Broth is recommended for detection and presumptive identification of S. pyogenes based on PYR hydrolysis (4). Todd Hewitt Broth Base (M313) acts as the basal medium to which substrate for PYR enzyme is added (3). Beef heart infusion and peptic digest of animal tissue provide nitrogenous nutrients. Dextrose is the carbohydrate serving as an energy source. Disodium phosphate serves as buffering agent and sodium chloride maintains osmotic balance. Chromogenic mixture provides substrate for PYR enzyme. After an incubation at 35-37°C for 18-24 hours, add 1 drop of PYR reagent (R043) directly to suspected surface growth on plate. Observe for colour change after 2 minutes. The chromogenic mixture is hydrolysed by S. pyogenes to L-pyrrolidone and b-naphthylamine. The PYR reagent reacts with b-naphthylamine to form a red coloured Schiffs Base indicating a positive reaction.

**Formula / Liter**

| Ingredients | Gms / Liter |
|---|---|
| Beef heart infusion from | 500.000 |
| Peptic digest of animal tissue | 20.000 |
| Dextrose | 2.000 |
| Sodium chloride | 2.000 |
| Disodium phosphate | 0.400 |
| Sodium carbonate | 2.500 |
| Chromogenic mixture | 0.100 |
| Final pH: 7.8 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

**Precautions**
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

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**Directions**

1.Suspend 37 grams in 1000 ml distilled water.
2.Heat if necessary to dissolve the medium completely.
3.Mix well and dispense as desired.
4.Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Quality Control Specifications**

| | |
|---|---|
| **Dehydrated Appearance** | Cream to yellow homogeneous free flowing powder |
| **Prepared Medium** | Light yellow coloured clear solution |
| **Reaction of 4.03% Solution** | pH : 7.8 ± 0.2 at 25°C |
| **Gel Strength** | Not Applicable |

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

| Sr. No. | Organism | Inoculum (CFU) | Growth | PYR (on addition of PYR reagent) |
|---|---|---|---|---|
| 1. | *Streptococcus pyogenes ATCC 19615* | 50-100 | luxuriant | positive, red colouration |
| 2. | *Enterococcus faecalis ATCC 29212* | 50-100 | luxuriant | positive, red colouration |
| 3. | *Escherichia coli ATCC 25922* | 50-100 | luxuriant | negative |
| 4. | *Streptococcus agalactiae ATCC 12386* | 50-100 | luxuriant | negative |

**Test Procedure**

1. The suspected specimen may be used directly for streaking or heat-treated or alcohol-treated specimens can be used for streaking. On incubation at 37°C for 24 hours colonies develop from 30-100% of the *B. anthracis* spores that would grow on non-selective Heart Infusion Agar (DM786), being smaller and smoother than on the later medium.
2. Refer appropriate references for standard test procedures.

**Results**

1. Colonies of *B. anthracis* appear in 36-40 hours after incubation at 37°C.
2. Roughly circular, creamy- white colonies with a ground-glass texture are further subcultured on blood agar plates for identification.
3. Capsule production can be seen directly or on blood agar plates.[4]
4. Refer appropriate references and procedures for interpretation of results.

**Storage**

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

**Expiration**

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

**Limitations of the Procedure**

1. PLET Agar Base inhibits growth of most strains of *B.cereus, B.subtilis*, other *Bacillus* species, *Enterobacteriaceae* and *Pseudomonas* species.

2. Some strains of *B. cereus* from soil form colonies but they are smaller than those of B. anthracis, minute after 24 hours and moderately sized after 48 hours.
3. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
4. Consult appropriate texts for detailed information and recommended procedures.

**Packaging**
**Product Name : PYR Broth**
**Product Code : DM1829**
**Available Pack sizes : 500gm**

**References**

1.Facklam R. R., Thacker L. G, Fox B., Eriquez L., 1982, J. Clin. Microbiol., 15 (6), a, 987-990.

2.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Edition, Lippinocott Williams and Wilkins, N.Y. 407-410.

3.Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C., Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed., J. B. Lippinccott Company. 4.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

Further Information

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