



PRODUCT SPECIFICATION SHEET

PLET Agar Base, Modified (DM1825)

Intended Use

PLET Agar Base, Modified (DM1825) is recommended for isolation and enumeration of *Bacillus anthracis*.

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*. PLET Agar Base, Modified Medium is a modification of the base medium with an increased concentration of EDTA, which helps in inhibiting *Staphylococcus aureus*.

Principles of the Procedure

PLET Agar Base, Modified contains beef heart infusion from solids and tryptose which provide carbonaceous and nitrogenous compounds required for growth. Sodium chloride helps to maintain the osmotic equilibrium of the medium. Thallous acetate and Polymyxin (MS195) are inhibitory agents allowing growth of *B. anthracis* while inhibiting contaminants. Lysozyme (MS195) particularly inhibits the growth of gram-negative contaminants.

Formula / Liter

Ingredients	Gms / Liter
Beef heart, infusion from	500.00
Tryptose	10.00
Sodium chloride	5.00
EDTA	0.35
Thallous acetate	0.04
Agar	15.00
Final pH: 7.3 ± 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.

Directions

1. Suspend 40.4 grams of the medium in 990 ml of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C. Aseptically add rehydrated contents of 1 vial of Anthracis Selective Supplement (MS195).
5. Mix well and dispense as desired.





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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.04% Solution	pH : 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Anthracis Selective Supplement (MS195), after an incubation at 35-37°C for 36-40 hours.

Sr. No.	Organisms	Results to be achieved
		Growth
1.	<i>Bacillus anthracis</i> ATCC 14578	good-luxuriant
2.	<i>Bacillus cereus</i> ATCC 10876	inhibited
3.	<i>Staphylococcus aureus</i> ATCC 25923	inhibited

The organisms listed are the minimum that should be used for quality control testing.

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, Modified 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.





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Packaging

Product Name : PLET Agar Base, Modified

Product Code : DM1825

Available Pack sizes : 500gm

References

1. Knisely R.F. 1966, J. Bacteriol, 92:784-786.
2. Norris J. R., Berkley C.W., Logan N.A., and O'Donnell A.G., 1981. In M. P. Starr et al (ed) The Prokaryotes : a Handbook on Habitats, Isolation and Identification of Bacteria, Vol. 2, Springer Verlag, Berlin.
3. Parry J.M., Turnbull P.C.B. and Gibson J.R., 1983, A Colour Atlas of Bacillus species. Wolfe Medical Publications, London, United Kingdom.
4. Murray et al, 1999, Manual of Clinical Microbiology, 7th Edition, ASM Press, Washington, D.C.

Further Information

For further information please contact your local MICROMASTER Representative.



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