



PRODUCT SPECIFICATION SHEET

Mycoplasma Agar Base (PPLO Agar Base) (DM174)

Intended Use

Mycoplasma Agar Base (PPLO Agar Base) (DM175) with the addition of enrichment it is used for isolation and cultivation of *Mycoplasma* species (*Pleuropneumonia* like organisms - PPLO).

Product Summary and Explanation

Among the members of class *Mollicutes*, *Mycoplasma* was first recognized from a case of pleuropneumonia in a cow. The organism was designated "pleuropneumonia-like organism," or PPLO.⁽¹⁾ Although some species are normal human respiratory tract flora, *M. pneumoniae* is a major cause of respiratory disease (primary atypical pneumonia, sometimes called "walking pneumonia").⁽¹⁾ *M. hominis*, *M. genitalium* and *Ureaplasma urealyticum* are important colonizers (and possible pathogens) of the human genital tract. PPLO (Mycoplasma) Agar was described by Morton, Smith and Leberman.⁽²⁾ It was used in a study of the growth requirements of *Mycoplasma*,⁽³⁾ along with the identification and cultivation of this organism.⁽⁴⁻⁶⁾ Pivotal information regarding *Mycoplasma* has been documented by Sabin.⁽⁷⁾ Hayflick et al have reported the information regarding the cultivation of *Mycoplasma*.⁽⁸⁾

Principles of the Procedure

Mycoplasma Agar Base contains beef heart infusion, peptic digest of animal tissue and peptone which provides nitrogen, vitamins, amino acids and carbon required for growth. Sodium chloride maintains the osmotic balance of these formulations. Many *Mycoplasma* require serum for their good growth and also presence of antibiotic is necessary to prevent the growth of contaminating organisms. Mostly the *Mycoplasma* species are aerobic or facultatively anaerobic but some are microaerophilic. Few are anaerobic saprophytic *Mycoplasma* which grow best at 22-35°C while pathogenic strains grow at 35°C. *Mycoplasma* when grow in the agar medium show typical morphology and form colonies below the agar surface and do not grow without serum.

Formula / Liter

Ingredients	Gms / Liter
Beef heart, infusion from	250.00
Peptic digest of animal tissue	10.00
Sodium chloride	5.00
Agar	15.00
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.
3. For the cultivation of *Mycoplasma* the medium ingredients and all the supplements should be free of any toxic substances even in small amounts.

Directions

1. Suspend 36 grams of the medium in 700 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 45°C and aseptically add 300 ml Horse serum (MS084) or 10 vials of Mycoplasma Enrichment Supplement (MS190).
5. Mix well before dispensing. 25% Ascitic fluid can be used instead of Horse serum.

Quality Control Specifications





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Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 3.6 % solution	pH 7.8 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed in presence of 10% Carbon dioxide with added ,1% Horse serum (MS084) or 10 vials of Mycoplasma Enrichment Supplement (MS190), after an incubation at 22-35°C for 48 hours.

Sr. No.	Organisms	Results to be achieved
		Growth
1.	<i>Mycoplasma bovis</i> ATCC 25523	good-luxuriant
2.	<i>Mycoplasma gallinarium</i> ATCC 19708	good-luxuriant
3.	<i>Mycoplasma pneumoniae</i> ATCC 15531	good-luxuriant
4.	<i>Streptococcus pneumoniae</i> ATCC 6303	good-luxuriant

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

Test Procedure

1. Inoculate the surface of plates containing the complete medium by adding drops of liquid inoculum or by a swab-inoculation technique.
2. Incubate plates at 35 ± 2°C for up to 21 days in a moist atmosphere containing 5-10% carbon dioxide or anaerobically if the presence of *M. buccale*, *M. faucium*, *M. orale* or *M. salivarium* is suspected.
3. Refer to appropriate references for standard test procedures.

Results

1. PPLO colonies are round with a dense center and a less dense periphery, resembling a "fried egg" on PPLO Agar. Vacuoles, large bodies characteristic of *Mycoplasma* species are seen in the periphery.
2. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.
3. Refer to appropriate references and standard test 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. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name: Mycoplasma Agar Base (PPLO Agar Base)

Product Code: DM175





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Available Pack sizes: 100gm / 500gm

References

1. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, Mo.
2. Morton, Smith and Leberman. 1951. Am. J. Syphilis Gonorrh. 35:361.
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4. Chanock, James, Fox, Turner, Mufso and Hayflick. 1962. Soc. Exp. Biol. Med. 110:884.
5. Craven, Wenzel, Calhoun, Hendley, Hamory and Gwaltney. 1976. J. Clin. Microbiol. 4:225.
6. Gregory and Cundy. 1970. Appl. Microbiol. 19:268.
7. Sabin, 1941, Bacteriol. Rev., 5:1, 331.
8. Hayflick and Chanock, 1965, Bacteriol. Rev., 29:185.

Further Information

For further information please contact your local MICROMASTER Representative.



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