# Technical Information

## PPLO Agar Base (Mycoplasma Agar Base)

## Product Code: DM 1266

**Application:** PPLO Agar Base (Mycoplasma Agar Base) with the addition of enrichment, is used for isolation and cultivation of *Mycoplasma* species (pleuropneumonia like organisms).

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Beef heart, infusion from | 250.000 |
| Peptic digest of animal tissue | 10.000 |
| Sodium chloride | 5.000 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 7.8±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Principle & Interpretation

Morton, Smith and Leberman [1] have described PPLO Agar in details and was used in a study of the growth requirements of *Mycoplasma,* along with the identification and cultivation of this organism [2-5]. Important information regarding *Mycoplasma* has been quoted by Sabin [6]. Hayflick et al have reported the information regarding the cultivation of *Mycoplasma* [7].

For the cultivation of *Mycoplasma* the medium ingredients and all the supplements should be free of any toxic substances even in using small amounts. Beef heart infusion, peptic digest of animal tissue and peptone provide nitrogen, vitamins, amino acids and carbon in these media. Sodium chloride maintains the osmotic balance of these formulations. Many *Mycoplasma* require serum for their good growth and addition 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 one grow at 35°C. *Mycoplasma* when grow in the agar medium show typical morphology and form colonies below the agar surface and do no grow in the absence of serum.

Plates or tubes should be incubated in an atmosphere containing 5-10% carbon dioxide and examined after incubation of 48 hours but they should not be discarded as negative until after incubation for 3 weeks.

PPLO colonies are round with a dense center and a less dense periphery, resembling a “fried egg” on PPLO Agar. Vacuoles, large body’s characteristic of *Mycoplasma* species are seen in the periphery. Colonies vary in diameter from 10 to 500 microns (0.0 1-0.5 mm) and penetrate into the medium.

## Methodology

Suspend 36 grams of powder media in 700 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C and aseptically add 300 ml Horse serum (MS3239) or 10 vials of Mycoplasma Enrichment Supplement (MS2075). Mix well before dispensing. 25% Ascitic fluid can be used instead of Horse serum.

## Quality Control

**Physical Appearance**
Cream to yellow homogeneous free flowing powder
**Gelling**
Firm, comparable with 1.5% Agar gel
**Colour and Clarity of prepared medium**
Yellow coloured clear to slightly opalescent gel forms in Petri plates
**Reaction**
Reaction of 3.6% w/v aqueous solution at 25°C. pH : 6.0±0.2
**pH Range** 7.60-8.00

**Cultural Response/ characteristices**

DM 1266: Cultural characteristics observed in presence of 10% Carbon dioxide with added ,1% Horse serum (MS3239) or 10 vials of Mycoplasma Enrichment Supplement(MS2075), after an incubation at 22-35°C for 48 hours.

| Organism | Growth |
|---|---|
| Mycoplasma bovis ATCC 25523 | good-luxuriant |
| Mycoplasma gallinarium ATCC 19708 | good-luxuriant |
| Mycoplasma pneumoniae ATCC 15531 | good-luxuriant |
| Streptococcus pneumoniae ATCC 6303 | good-luxuriant |

## Storage and Shelf Life

**Dried Media:** Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
**Prepared Media**: 2-8$^{o}$ in sealable plastic bags for 2-5 days.

## Further Reading

1. Morton, Smith and Leberman, 1951, Am. J. Syphilis Gonorrh. Veneral Diseases, 35: 361.
2. Morton and Lecce, 1953. J. Bacteriol., 66:646.
3. Chanock, James, Fox, Turner, Mufso and Hayflick, 1962, Soc. Exp. Biol. Med., 110:884.
4. Craven, Wenzel, Calhoun, Hendley, Hamory and Gwaltney, 1976, J. Clin. Microbiol., 4:225.
5. Gregory and Cundy, 1970, Appl. Microbiol., 19:268.
6. Sabin, 1941, Bacteriol. Rev., 5:1, 331.
7. Hayflick and Chanock, 1965, Bacteriol, Rev., 29:185.

## Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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