



TECHNICAL DATA SHEET

AS-1333

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PPLO Broth (Mycoplasma Broth Base)

PPLO Broth Base without Crystal Violet

Document Control

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Technical Review

Hassan Salimi, Technical Director, AuSaMicS Pty Ltd (18+ years culture media formulation)

Product Identification

Product Name	PPLO Broth (Mycoplasma Broth Base)
Synonyms	PPLO Broth Base without Crystal Violet
Catalogue Number	AS-1333
Format	Dehydrated powder
Manufacturer	AuSaMicS Pty Ltd, Melbourne, Australia

Intended Use

Basal medium for the isolation and cultivation of Mycoplasma species (pleuropneumonia-like organisms), requiring aseptic supplementation with serum and yeast extract to become a complete growth medium.

Mode of Action

Beef Heart Infusion and Peptone supply amino acids and peptides required for Mycoplasma growth. Sodium Chloride maintains osmotic equilibrium, particularly important given Mycoplasma's lack of a rigid cell wall. The base alone is nutritionally incomplete for Mycoplasma — these organisms require exogenous cholesterol and other membrane precursors, supplied by serum supplementation, since their minimal genome cannot synthesise these components.

Formulation (per litre)

Ingredient	Quantity	Function
Beef Heart Infusion (from 50g fresh tissue)	6.0 g	Nutrient source for Mycoplasma growth
Peptone	10.0 g	Amino acid / nitrogen source
Sodium Chloride	5.0 g	Osmotic stabiliser

Total: 21.0 g/L Final pH: 7.8 ± 0.2 at 25°C. Contains neither crystal violet nor phenol red.

Formulation verified against HiMedia (M1586) and Neogen (NCM0113A) technical specifications, which independently agree on total weight (21.0 g/L) and composition. Both confirm the prepared medium is yellow-coloured and clear — not pink or red.



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Preparation

- Suspend 21.0 g of dehydrated medium in 700 mL purified water
- Heat to boiling to dissolve the medium completely
- Sterilise by autoclaving at 121°C for 15 minutes
- Cool to 45–50°C and aseptically add 300 mL sterile horse serum (or equivalent enrichment supplement)
- Mix well and dispense into sterile tubes or flasks as required

Supplementation Guide

- Horse Serum: aseptically add after autoclaving and cooling — reference procedure uses 300 mL per 700 mL base (30% v/v of final volume); ratios in the 10–30% v/v range are reported across different sources.
- Yeast Extract: a fresh 25% w/v yeast extract solution is commonly added alongside serum to supply B-complex vitamins and enhance growth.
- Selective agents (optional): penicillin, thallium acetate, or amphotericin B may be added to suppress bacterial or fungal contaminants where a selective formulation is required.
- This base medium as supplied does not include serum, yeast extract, or antibiotics — all must be added by the user according to their validated protocol.

Incubation Conditions

Parameter	Condition
Temperature	35°C (22–35°C for saprophytic strains)
Time	Up to 30 days; do not discard as negative before this period
Atmosphere	5–10% CO ₂ (most strains); some anaerobic saprophytic strains

Quality Control — Cultural Response (Supplemented Medium)

Organism	ATCC No.	Expected Result
<i>Mycoplasma bovis</i>	25523	Good to luxuriant growth
<i>Mycoplasma pneumoniae</i>	15531	Good to luxuriant growth
<i>Escherichia coli</i>	25922	Inhibited (differential control)
<i>Staphylococcus aureus</i>	25923	Inhibited (differential control)

Storage & Shelf Life

Dehydrated medium

10–30°C, dry, tightly sealed — 24 months



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Prepared medium

2–8°C, protected from light

Limitations & Technical Considerations

- Base medium only — will not support Mycoplasma growth without serum and yeast extract supplementation added by the user.
- No visual growth indicator — since the base contains no phenol red, colour-change monitoring requires either adding an indicator yourself or subculturing to agar for colony confirmation.
- Slow growth — some Mycoplasma species require up to 30 days incubation.
- Ureaplasma urealyticum may be inhibited at this medium's pH; a lower-pH modification is typically used for Ureaplasma recovery.

Literature & References

- Morton, H.E., Lecce, J.G. (1953). Journal of Bacteriology, 66:646.
- Morton, H.E., Smith, P.F., Leberman, P.R. (1951). American Journal of Syphilis, Gonorrhea and Venereal Diseases, 35:361.
- Hayflick, L., Chanock, R.M. (1965). Mycoplasma Species of Man. Bacteriological Reviews, 29(2). American Society for Microbiology.

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