AuSaMicS™ Life Science

PRODUCT DESCRIPTION

AS-1339 | Reinforced Clostridial Medium (RCM)

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Reinforced Clostridial Medium (RCM)

Enriched Reducing Semi-Solid Medium for Cultivation and Enumeration of Clostridia and Anaerobes

Catalogue Number: AS-1339

Application	Pharmacopoeial	Final pH	HS Code
Clostridium & anaerobe culture	EP / USP / JP harmonised	6.8 ± 0.2 at 25 °C	3821.00.00

Overview

Reinforced Clostridial Medium (RCM) was originally proposed by Hirsch and Grinsted (1954) for the cultivation and enumeration of *Clostridia*, other obligate anaerobes, and facultative microorganisms from foodstuffs and environmental materials. The medium has since become an international pharmacopoeial standard, with current specifications harmonised across the European Pharmacopoeia (EP 2.6.13), United States Pharmacopoeia (USP <62>), and Japanese Pharmacopoeia (JP Chapter 4.05) for Microbial Examination of Non-Sterile Products.

The medium is nutritionally rich — combining meat extract, peptone, and yeast extract — and contains L-cysteine hydrochloride as a reducing agent and sodium acetate as a buffer, together providing conditions favourable for obligate anaerobic growth. The low agar concentration (0.5 g/L) creates a semi-solid consistency that retards oxygen diffusion without forming a solid gel, allowing anaerobic zones to develop at depth.

Mode of Action

RCM is free from selective inhibitors. **L-Cysteine hydrochloride** is the primary reducing agent, scavenging dissolved oxygen and maintaining a low oxidation-reduction potential (Eh) suitable for obligate anaerobes. **Sodium acetate** acts as a pH buffer and supplementary carbon source. **Soluble starch** absorbs toxic metabolic byproducts. The semi-solid agar forms an oxygen gradient — anaerobic conditions at depth support obligate anaerobes, while the upper zone permits facultative anaerobe and aerobe recovery. Polymyxin B (not included) may be added post-sterilisation at 0.02 g/L to selectively inhibit Gram-negative organisms if required.

Typical Composition (per litre)

Ingredient	CAS Number	Function	Amount (g/L)
Meat Extract	8001-31-8	Rich source of meat peptides, amino acids, glycogen, growth factors	10.0

Peptone (Enzymatic Digest)	73049-73-7	Primary nitrogen and carbon source — amino acids and peptides	10.0
Yeast Extract	8013-01-2	B-vitamins, nucleotides, coenzymes, growth factors	3.0
D(+)-Glucose	50-99-7	Fermentable carbohydrate — energy source; aids anaerobic metabolism	5.0
Soluble Starch	9005-84-9	Absorbs toxic metabolic byproducts; protective colloid	1.0
Sodium Chloride (NaCl)	7647-14-5	Osmotic balance	5.0
Sodium Acetate ($C_2H_3NaO_2$)	127-09-3	pH buffer; supplementary carbon source for acetoclastic organisms	3.0
L-Cysteine Hydrochloride	52-89-1	Primary reducing agent — scavenges O_2, lowers Eh	0.5
Agar (semi-solid)	9002-18-0	Retards O_2 diffusion; creates anaerobic gradient at depth	0.5
Purified Water	7732-18-5	Solvent	q.s. 1 L

Total (without water): 38.0 g/L | Final pH: 6.8 ± 0.2 at 25 °C

Key Features

- Pharmacopoeial harmonised — complies with EP 2.6.13, USP <62>, JP Chapter 4.05
- Rich nutrient base — meat extract + peptone + yeast extract + glucose supports fastidious anaerobes
- L-Cysteine reducing agent — effective O_2 scavenger without the colour interference of thioglycollate
- Semi-solid agar (0.5 g/L) — oxygen gradient without solidification; anaerobic at depth
- Starch — absorbs toxic metabolic byproducts from anaerobic fermentation
- No selective inhibitors — broad recovery of both strict and facultative anaerobes
- Optional Polymyxin B supplementation — suppresses Gram-negative background if required
- Compatible with paraffin or agar overlay for strict anaerobic applications

Applications

Pharmaceutical Quality Control

- Microbial examination of non-sterile pharmaceutical products for specified microorganisms — EP 2.6.13, USP <62>, JP Chapter 4.05

- Detection and enumeration of Clostridium sporogenes, C. perfringens, C. novyi in pharmaceutical raw materials and finished products
- Validation of antimicrobial effectiveness testing

Food Microbiology

- Enumeration of anaerobic sporeformers (Clostridia) in cheese, dairy, and processed foods — original application (Hirsch & Grinsted, 1954)
- Detection of gas-producing Clostridia in canned and vacuum-packed foods
- Spoilage organism investigation in anaerobic food environments

Clinical & Research Microbiology

- Enrichment and cultivation of clinical Clostridia from wound, faecal, and blood specimens
- Propagation and maintenance of obligate anaerobe reference strains
- Comparative anaerobe physiology studies

Preparation Instructions

1. Dissolve 38.0 g of dehydrated RCM in 1 litre of purified water.
2. Heat with agitation until completely dissolved. Do not boil excessively.
3. Dispense into test tubes (8–10 mL per tube) or bottles as required.
4. Sterilise by autoclaving at 121 °C for 15 minutes.
5. Cool to room temperature. The prepared medium should appear clear and yellowish.
6. Optional — Polymyxin B: add 0.02 g/L in sterile aqueous solution post-autoclaving under aseptic conditions to inhibit Gram-negative organisms.
7. For strict anaerobic applications: overlay inoculated medium with sterile paraffin viscous (2–3 mL) or 1.5% agar layer to exclude oxygen.

For strict obligate anaerobe work: reduce dissolved oxygen by heating medium in a boiling water bath for 15 minutes and cooling rapidly before inoculation. Inoculate immediately.

Incubation & Interpretation

Temperature	30–35 °C (standard); 35–37 °C for clinical Clostridia
Duration	48 h (standard); up to 5–7 days for slow-growing or spore-forming anaerobes
Atmosphere	Anaerobic — use anaerobic jar, glove box, or paraffin/agar overlay

Reading	Turbidity or pellicle formation indicates growth; gas production may be visible

Pharmacopoeial note: For EP/USP/JP compliance testing, follow the exact inoculation volumes, incubation temperatures, and reading intervals specified in the applicable pharmacopoeial method.

Storage & Stability

Dehydrated powder	15–25 °C, tightly sealed, dry, original container, protected from light and moisture
Prepared tubes / bottles	15–25 °C upright, sealed, protected from light; use within 3 weeks
Shelf life (powder)	As per labelled expiry date
Do not use if	Turbidity before inoculation, colour change, or precipitate observed

Customs & Trade Information

HS / AHECC Code	3821.00.00
Description	Prepared culture media for development or maintenance of microorganisms
Country of Origin	Australia

Literature & References

Hirsch, A. & Grinsted, E. (1954). Methods for the growth and enumeration of anaerobic sporeformers from cheese, with observations on the effect of nisin. Journal of Dairy Research, 21, 101–110.

European Pharmacopoeia (EP) 2.6.13 — Microbiological examination of non-sterile products: Tests for specified microorganisms.

United States Pharmacopoeia USP <62> — Microbiological examination of non-sterile products: Tests for specified microorganisms.

Japanese Pharmacopoeia, Chapter 4.05 — Microbial Limit Test II.

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