MUELLER HINTON AGAR

Dehydrated Powder · AST Reference Medium · Kirby–Bauer / CLSI / EUCAST

Catalog No.	HS Code	Form	Grade
AS-1299	**3821.00.00**	**Dehydrated Powder**	**AST Reference**

Issue Date	Version	Review Date	Document No.
01 January 2025	**v1.0 — Initial Issue**	**01 January 2026**	**TDS-AS1299-v1.0**

1. PRODUCT IDENTIFICATION

Product Name	Mueller Hinton Agar (MHA) Powder
Catalog Number	AS-1299
HS / AHECC Code	3821.00.00 — Prepared culture media for development or maintenance of microorganisms
Physical Form	Dehydrated powder — light beige to cream
Grade	Microbiology / Antimicrobial Susceptibility Testing (AST) Reference Medium
Regulatory Ref.	CLSI M02 / M07 · EUCAST · ISO 20776-1 · WHO / FDA recommended
Supplier	Ausamics Pty Ltd · 31 Longview Ct, Thomastown VIC 3074, Australia
Contact	+61 412 520 598 · support@Ausamics.com · Ausamics.com.au

2. PRODUCT OVERVIEW & BACKGROUND

Standard Medium. Mueller Hinton Agar (MHA) is the internationally mandated reference solid medium for antimicrobial susceptibility testing (AST) of non-fastidious bacteria. Originally described by Mueller and Hinton in 1941, it was subsequently adopted as the sole recommended medium for the Kirby–Bauer disk diffusion method by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Why MHA. MHA's controlled composition makes it uniquely suited for AST. Most culture media are inappropriate for susceptibility testing because they contain variable amounts of thymidine (which antagonises folate inhibitors), uncontrolled cation levels (which affect aminoglycoside and tetracycline MICs), and inconsistent agar depth — all of which introduce systematic errors into inhibition zone measurements.

Critical Parameters. The three most critical quality parameters in MHA are: (1) low thymidine / thymine content for sulfonamide and trimethoprim testing, (2) controlled calcium and magnesium ion concentrations for aminoglycoside and polymyxin reproducibility, and (3) consistent agar depth of 4.0 ± 0.5 mm to ensure uniform antibiotic diffusion gradients.

Starch Function. The starch component serves a dual purpose: it absorbs bacterial inhibitory substances (toxins, fatty acids) that might otherwise diffuse outward and create false inhibition zones, and it provides a clear, pale agar matrix that allows unambiguous visual reading of zone edges against a plain background.

REGULATORY STATUS Mueller Hinton Agar AS-1299 is formulated to meet the performance requirements of CLSI M02/M07, EUCAST, and ISO 20776-1. A Certificate of Analysis is issued for every batch confirming compliance with specified cation concentrations, thymidine content, and growth promotion / inhibition zone reproducibility.

3. APPLICATIONS

AST METHODS

- Kirby–Bauer disk diffusion (standard method)
- Minimum Inhibitory Concentration (MIC) agar dilution
- Gradient diffusion (Etest / MIC strip) testing
- Antimicrobial resistance surveillance studies
- Combination antibiotic synergy testing
- Breakpoint determination experiments

TEST ORGANISMS

- *Staphylococcus aureus (incl. MRSA)*
- *Escherichia coli*
- *Pseudomonas aeruginosa*
- *Acinetobacter spp.*
- *Enterococcus spp.*
- *Enterobacteriaceae family*
- *Klebsiella pneumoniae*
- *Proteus mirabilis*

MODIFIED VARIANTS

- MHA + 5% defibrinated blood → Streptococcus spp.
- MH-F (with supplements) → Haemophilus influenzae
- MHA + 2% NaCl → MRSA / oxacillin screening
- CAMHB (broth) → MIC broth microdilution
- MHA + lysed horse blood → Fastidious organisms

4. COMPOSITION & SPECIFICATIONS

Typical Composition — per litre of prepared medium

Ingredient	Amount	Function
Acid Hydrolysate of Casein	**17.5 g**	Amino acids & peptides — primary nitrogen source
Beef Infusion Solids	**2.0 g**	Growth factors, B vitamins, trace elements
Starch	**1.5 g**	Toxin absorption; clear zone definition
Bacteriological Agar	**17.0 g**	Solidifying agent — inert matrix
Final pH at 25°C	**7.3 ± 0.1**	Critical — affects diffusion & zone size

Controlled Cation Content — after sterilisation

Ion	Required Concentration	Clinical Significance
Calcium (Ca^{2+})	**20–25 mg/L**	Affects aminoglycoside MICs vs. Pseudomonas; tetracycline activity
Magnesium (Mg^{2+})	**10–12.5 mg/L**	Regulates polymyxin and aminoglycoside susceptibility results

CRITICAL PARAMETER Low thymidine and thymine content is essential for valid sulfonamide and trimethoprim susceptibility testing. Excess thymidine causes false resistance results by bypassing the folate inhibition mechanism.

5. METHOD OF PREPARATION

Step	Action	Parameters / Notes
1	Suspend 38.0 g of Mueller Hinton Agar powder in 1 litre of purified (distilled or Type 2) water	Do not use tap water — mineral content varies and may affect cation levels
2	Heat with frequent agitation and boil for 1 minute to dissolve completely	Ensure complete dissolution — undissolved particles indicate incomplete hydration
3	Sterilize by autoclaving at 121°C for 15 minutes	Do not over-autoclave — prolonged heating degrades thymidine content and alters pH
4	Cool to 45–50°C in a water bath before pouring	Do not allow to cool below 44°C — agar will solidify in the flask
5	Pour 25–30 mL per 90–100 mm Petri dish on a level surface	CRITICAL: Final agar depth must be 4.0 ± 0.5 mm — use a calibrated pour or volume-controlled dispenser
6	Allow to solidify at room temperature, then dry plates in a 37°C incubator (10–15 min, lids open) or laminar flow hood	Excess surface moisture causes zone spreading and inaccurate readings
7	Inspect plates: smooth, clear, amber-coloured agar. No bubbles, cracks, or contamination.	Discard any plates with visible defects — they will give inconsistent zone results
8	Store prepared plates at 2–8°C in sealed plastic bags until use	Use within 7 days of preparation. Equilibrate to room temperature before use.

AGAR DEPTH — CRITICAL The agar depth of 4.0 ± 0.5 mm is specified by CLSI and EUCAST as mandatory for disk diffusion testing. Shallow plates give falsely large zones; deep plates give falsely small zones. Verify depth by measuring with a calibrated depth gauge on multiple plates from each pour batch.

6. INCUBATION CONDITIONS

Temperature	35 ± 1°C (33–35°C for MRSA oxacillin / methicillin testing)
Atmosphere	Ambient air — do NOT incubate in CO_2 for standard AST (CO_2 lowers pH and affects zone sizes)

Incubation Time	16–20 hours for most organisms. Read at exactly 16–18 h for staphylococci.
MRSA / NaCl plates	Incubate at 33–35°C for a full 24 hours before reading oxacillin zones
Inoculum Standard	0.5 McFarland turbidity standard (1–2 × 10^8 CFU/mL) — calibrate photometrically
Inoculation Method	Flood or 3-direction streaking within 15 minutes of inoculum preparation
Disk Application	Apply disks within 15 minutes of inoculation. Press firmly with sterile forceps — disks must contact agar.
Zone Reading	Measure zone of complete inhibition (including disk diameter) in mm using ruler or digital caliper

7. QUALITY CONTROL — REFERENCE ORGANISMS & EXPECTED ZONE RANGES

QC REQUIREMENT Susceptibility testing results are only valid if QC organism zone diameters fall within the ranges specified below. Run QC organisms with every batch of plates and each new lot of antibiotic disks.

QC Organism	ATCC No.	Antibiotic (Disk)	Expected Zone (mm)
Staphylococcus aureus	25923	Oxacillin (1 µg)	**18–24 mm**
Staphylococcus aureus	25923	Ampicillin (10 µg)	**27–35 mm**
Escherichia coli	25922	Ampicillin (10 µg)	**16–22 mm**
Escherichia coli	25922	Ciprofloxacin (5 µg)	**30–40 mm**
Pseudomonas aeruginosa	27853	Gentamicin (10 µg)	**16–21 mm**
Pseudomonas aeruginosa	27853	Piperacillin (100 µg)	**25–33 mm**
Staphylococcus aureus	29213	Penicillin (10 U)	**26–37 mm**

** Zone ranges based on CLSI M100 performance standards. Verify against current edition of CLSI M100 or EUCAST disk diffusion breakpoint tables for your specific lot of antibiotic disks.*

8. TROUBLESHOOTING GUIDE

Problem	Likely Cause(s)	Corrective Action
QC zones outside acceptable range	Wrong agar depth; incorrect inoculum; pH out of range; disk potency expired	Verify depth (4±0.5 mm); calibrate 0.5 McFarland; check plate pH; replace disk cartridges
Zones too large (all antibiotics)	Agar depth < 3.5 mm; inoculum too light; over-dried agar surface	Pour to correct volume; use photometric McFarland calibration; reduce drying time

Problem	Likely Cause(s)	Corrective Action
Zones too small (all antibiotics)	Agar depth > 4.5 mm; inoculum too heavy; agar not equilibrated to room temp	Correct pour volume; dilute inoculum to 0.5 McFarland; pre-warm plates to 35°C
Skewed or elliptical zones	Plate not level during pour; uneven agar depth; disk not flat on agar	Use levelling table; verify depth at multiple points; press disks firmly
Confluent growth / no inhibition zones	Inoculum too heavy; disk out of potency; resistant organism selected	Verify 0.5 McFarland standard; replace disks; check organism identity
Haze inside inhibition zone	Trimethoprim / sulfonamide testing with high-thymidine agar or blood	Use only MHA verified for low thymidine; do not add blood for TMP/SMX testing
Agar bubbles or surface defects	Overheating or boiling during dissolving; too rapid pour	Degas medium at 45°C before pouring; reduce agitation speed
Poor growth of test organism	Inoculum deteriorated; plates expired; inappropriate incubation	Prepare fresh inoculum from overnight culture; use plates within 7 days

9. PHYSICAL & CHEMICAL PROPERTIES

Physical State	Dehydrated powder
Appearance	Light beige to cream homogeneous powder
Odour	Slight, characteristic meat/casein odour
Reconstitution Rate	38.0 g per litre of purified water
Prepared Medium	Clear to slightly opaque amber-coloured agar
Final pH (25°C)	7.3 ± 0.1 — measured on cooled, prepared agar
Agar Gel Strength	Suitable for disk diffusion — standard bacteriological grade
Solubility	Readily dispersible and soluble upon boiling in water
Sterilisation	Autoclave 121°C / 15 minutes — do not filter sterilise
Loss on Drying	≤ 5.0% w/w at 105°C / 2 hours
Calcium Content	20–25 mg/L in prepared medium
Magnesium Content	10–12.5 mg/L in prepared medium
Thymidine Content	Low — verified per batch (critical for sulfonamide / trimethoprim testing)

10. QUALITY CONTROL & BATCH RELEASE

BATCH RELEASE Every lot of Mueller Hinton Agar AS-1299 is tested before release. A Certificate of Analysis (COA) confirming all specifications below is available for every batch on request from support@Ausamics.com.

Test Parameter	Method	Specification	Result
Appearance	Visual inspection	Cream-beige powder	✓ Complies
pH (prepared)	Calibrated pH meter, 25°C	7.3 ± 0.1	✓ Complies
Calcium (Ca^{2+})	ICP-OES or colorimetric	20–25 mg/L	✓ Complies
Magnesium (Mg^{2+})	ICP-OES or colorimetric	10–12.5 mg/L	✓ Complies
Thymidine content	Microbiological assay	Low — meets CLSI criteria	✓ Complies
Loss on drying	Gravimetric, 105°C/2h	≤ 5.0% w/w	✓ Complies
Gel strength	Bloom gelometer	Suitable for disk diffusion	✓ Complies
Growth promotion	E. coli ATCC 25922 zone	16–22 mm (ampicillin 10µg)	✓ Complies
QC S. aureus	ATCC 25923 zone	27–35 mm (ampicillin 10µg)	✓ Complies
Sterility	USP <71> / ISO 11737	No growth	✓ Complies

11. STORAGE & STABILITY

Dehydrated Powder — Temp.	+15°C to +30°C (cool, dry location)
Dehydrated Powder — Humidity	≤ 60% RH · Keep sealed · Hygroscopic — moisture causes caking and pH shift
Dehydrated Powder — Container	Original, tightly sealed container. Re-seal immediately after each use.
Powder Shelf Life	36 months from manufacture date when stored correctly. Refer to label for expiry.
Prepared Agar Plates — Temp.	+2°C to +8°C in sealed plastic bags
Prepared Plates — Shelf Life	7 days maximum under routine laboratory conditions
Prepared Plates — Use	Equilibrate to room temperature before use. Dry surface moisture under laminar flow or in 37°C incubator (lids ajar, 10–15 min) before inoculation.
Stability Indicator	Prepared agar: clear, pale amber, no visible contamination, no syneresis (weeping). Discard any plates with surface defects.

12. PACKAGING & ORDERING INFORMATION

Pack Size	Catalog No.	Plates Yield (approx.)	Suitable For
100 g	AS-1299-100	~70 plates	Evaluation / small lab
500 g	AS-1299	~350 plates	Routine clinical laboratory

Pack Size	Catalog No.	Plates Yield (approx.)	Suitable For
2 kg	AS-1299-2K	~1,400 plates	High-volume / hospital lab
5 kg	AS-1299-5K	~3,500 plates	Reference / bulk laboratory
Bulk	AS-1299-BLK	Custom	Commercial / OEM / industrial

** Plate yield based on 28 mL per 90 mm plate. Actual yield depends on pour volume. Contact Ausamics for bulk pricing and custom quantities.*

13. RELATED PRODUCTS & ACCESSORIES

Blood Agar Base	Ausamics Cat. No. AS-1105 · For MHA + blood supplemented variants
Cation-Adjusted MHB (Broth)	Ausamics Cat. No. AS-1300 · For broth microdilution MIC testing (CAMHB)
McFarland Standard Kit (0.5)	Ausamics Cat. No. AQC-050 · For inoculum standardisation
Antibiotic Disk Dispensers	Available through Ausamics — Oxoid and Mast compatible formats
Petri Dishes (90 mm, vented)	Ausamics Cat. No. ALD-090 · Sterile, individually wrapped
Certificate of Analysis	Available per lot on request · Email: support@Ausamics.com
Safety Data Sheet (SDS)	SDS-AS1299-v1.0 · Available at Ausamics.com.au or on request

14. REGULATORY & COMPLIANCE INFORMATION

Intended Use	For laboratory antimicrobial susceptibility testing only. Not for human, veterinary, food, feed, or pharmaceutical use.
Standards Compliance	CLSI M02 (disk diffusion) · CLSI M07 (MIC dilution) · EUCAST disk diffusion standard · ISO 20776-1
Regulatory Support	Performance meets criteria specified by FDA, WHO, and TGA for susceptibility testing media
AICIS Status	Exempt from industrial chemical registration for laboratory use (Industrial Chemicals Act 2019, Australia)
HS / AHECC Code	3821.00.00 — Prepared culture media for development or maintenance of microorganisms
Quality Management	Manufactured under controlled conditions with full batch documentation and QC release testing
Document Control	TDS-AS1299-v1.0 · Issue Date: 01 January 2025 · Review: 01 January 2026

DISCLAIMER The information in this Technical Data Sheet is based on data considered accurate at the time of issue. Ausamics Pty Ltd does not warrant completeness or accuracy for all applications. Users are responsible for validating the product for their

specific use and for compliance with applicable regulations and standards, including current editions of CLSI, EUCAST, and ISO documents. Ausamics Pty Ltd's liability is limited to the purchase price of the product supplied.

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31 Longview Ct, Thomastown VIC 3074 · ABN 56 676 640 467 · Ausamics.com.au