TECHNICAL DATA SHEET
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Bushnell-Haas Agar

Bushnell-Haas Medium | Hydrocarbon-Degrading Microorganism Medium

Document Control	Version 1.0 · Reviewed 31 August 2025
Technical Review	Hassan Salimi, Technical Director, AuSaMicS Pty Ltd (18+ years culture media formulation)

Product Identification

Product Name	Bushnell-Haas Agar
Synonyms	Bushnell-Haas Medium
Catalogue Number	AS-1161
HS Code	3821.00
Format	Dehydrated powder
Manufacturer	AuSaMicS Pty Ltd, Melbourne, Australia

Intended Use

Carbon-free mineral salts agar for the isolation, enrichment, and study of hydrocarbon-degrading microorganisms in soil, water, fuel, and oil-impacted samples.

Mode of Action

The formulation supplies nitrogen, phosphorus, and trace elements (magnesium, calcium, iron) but deliberately contains no added carbon source. Agar solidifies the medium for plate-based isolation. Only organisms able to metabolise an externally supplied hydrocarbon as their sole carbon and energy source can form colonies.

Formulation (per litre)

Ingredient	Quantity	Function
Magnesium Sulfate (MgSO4.7H2O)	0.20 g	Trace nutrient, magnesium source
Calcium Chloride, anhydrous (CaCl2)	0.02 g	Trace nutrient, calcium source
Monopotassium Phosphate (KH2PO4)	1.00 g	Phosphorus source, buffering
Dipotassium Phosphate (K2HPO4)	1.00 g	Phosphorus source, buffering
Ammonium Nitrate (NH4NO3)	1.00 g	Nitrogen source
Ferric Chloride (FeCl3.6H2O)	0.05 g	Trace nutrient, iron source
Agar	20.00 g	Solidifying agent

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Total: 23.27 g/L **Final pH: 7.0 ± 0.2 at 25°C. Contains no carbon source — supplied by the user as a hydrocarbon supplement.**

> *Calcium chloride is anhydrous (CaCl2), consistent with the original Bushnell and Haas (1941) formula and current HiMedia / peer-reviewed technical documentation — not the dihydrate form at the same mass.*

Preparation

- Suspend 23.27 g in 1 L distilled or purified water
- Heat with gentle agitation and bring to boiling until completely dissolved
- Dispense into suitable containers
- Sterilise by autoclaving at 121°C for 15 minutes
- Cool and pour plates

Incubation Conditions

Parameter	Condition
Temperature	25–30°C
Time	Up to 1–2 weeks (hydrocarbon-degrading organisms may grow slowly)
Atmosphere	Aerobic
Endpoint	Colony formation on hydrocarbon-supplemented plate

Quality Control — Cultural Response

Organism / Control	ATCC No.	Test Condition	Expected Result
Pseudomonas aeruginosa	27853	Plate + sterile mineral oil overlay, inoculated	Colony growth within 1 week at 25–30°C
Uninoculated control	—	Plate + mineral oil, no inoculum	No growth (sterility control)
Negative growth control	—	Inoculated, no hydrocarbon added	No significant growth (confirms selectivity)

Working With Hydrocarbon Supplements

- The hydrocarbon is not supplied as part of the dehydrated medium and must be sourced separately.
- Liquid hydrocarbons (mineral oil, kerosene, diesel, paraffin) are typically layered onto the surface of inoculated agar.
- Volatile hydrocarbons (e.g., gasoline) are often added to the lid of an inverted plate.
- Always add hydrocarbon supplements aseptically and follow the substrate's own SDS and risk controls.

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Research Design Notes

Growth on this medium is best interpreted as one line of evidence for hydrocarbon-utilisation capability, not proof of complete degradation. For biodegradation claims, pair microbiological growth with an analytical measurement of substrate depletion (e.g., GC-FID) or metabolite formation. Recommended controls include a no-carbon inoculated control, an abiotic substrate control, and a positive growth control in a separate suitable medium.

Storage & Shelf Life

Dehydrated medium	10–30°C, tightly closed, protected from moisture/light — 24 months
Prepared plates	2–8°C, inverted — use within 2 weeks

Limitations & Technical Considerations

- Requires a hydrocarbon supplement — the base agar alone will not support growth.
- Growth does not prove complete degradation — indicates utilisation capability only.
- Extended incubation may be needed — 1–2 weeks at 25–30°C is common.
- Yellow-orange precipitate after autoclaving is normal and not indicative of contamination.

Literature & References

- Bushnell, L.D., Haas, H.F. (1941). The utilization of certain hydrocarbons by microorganisms. Journal of Bacteriology, 41(5), 653–673. DOI: 10.1128/jb.41.5.653-673.1941.
- SIM Committee on Microbiological Deterioration of Fuels. Recommended procedures for the microbiological examination of fuels.