



TECHNICAL DATA SHEET

AS-1162

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Bushnell-Haas Broth

Bushnell-Haas Medium | Hydrocarbon-Degrading Microorganism Medium

Document Control

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Technical Review

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

Product Identification

Product Name	Bushnell-Haas Broth
Synonyms	Bushnell-Haas Medium
Catalogue Number	AS-1162
HS Code	3821.00
Format	Dehydrated powder
Manufacturer	AuSaMicS Pty Ltd, Melbourne, Australia

Intended Use

Carbon-free mineral salts medium for the isolation, enumeration, and study of hydrocarbon-degrading microorganisms in soil, water, fuel, and oil-spill samples, and for microbiological quality control of petroleum-based products.

Mode of Action

The formulation supplies nitrogen, phosphorus, and trace elements (magnesium, calcium, iron) but deliberately contains no carbon source. Only organisms able to metabolise an externally supplied hydrocarbon (e.g., mineral oil, kerosene, diesel, or paraffin) as their sole carbon and energy source can grow. Growth is observed as turbidity in the liquid medium, which serves as the diagnostic endpoint without need for additional indicators.

Formulation (per litre)

Ingredient	Quantity	Function
Magnesium Sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0.20 g	Trace nutrient, magnesium source
Calcium Chloride, anhydrous (CaCl_2)	0.02 g	Trace nutrient, calcium source
Monopotassium Phosphate (KH_2PO_4)	1.00 g	Phosphorus source, buffering
Dipotassium Phosphate (K_2HPO_4)	1.00 g	Phosphorus source, buffering
Ammonium Nitrate (NH_4NO_3)	1.00 g	Nitrogen source
Ferric Chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$)	0.05 g	Trace nutrient, iron source



TECHNICAL DATA SHEET

AS-1162

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Total: 3.27 g/L Final pH: 7.0 ± 0.2 at 25°C. Contains no carbon source — supplied by the user as a hydrocarbon supplement.

Preparation

- Suspend 3.27 g in 1 L distilled or purified water
- Heat gently with frequent agitation until completely dissolved
- Dispense into tubes or flasks
- Sterilise by autoclaving at 121°C for 15 minutes
- Cool, then aseptically add a sterile hydrocarbon source before or after inoculation

A white precipitate before sterilisation becoming yellow to orange after autoclaving is a normal characteristic of this medium and does not affect performance.

Incubation Conditions

Parameter	Condition
Temperature	25–30°C
Time	Up to 1–2 weeks (hydrocarbon-degrading organisms may grow slowly)
Atmosphere	Aerobic
Endpoint	Turbidity in liquid culture indicates hydrocarbon-degrading growth

Quality Control — Cultural Response

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

Working With Hydrocarbon Supplements

- Liquid hydrocarbons (mineral oil, kerosene, diesel) are typically layered onto the surface of inoculated broth or agar.
- Volatile hydrocarbons (e.g., gasoline) are often added to the lid of an inverted plate rather than directly into the medium.
- Always add hydrocarbon supplements aseptically and work in a well-ventilated area or fume hood.
- Dispose of hydrocarbon-contaminated materials according to laboratory biosafety and chemical waste procedures.



TECHNICAL DATA SHEET

AS-1162

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Storage & Shelf Life

Dehydrated medium	10–30°C, tightly closed, protected from moisture/light — 24 months
Prepared medium	2–8°C — use within 2 weeks; discard if turbid, contaminated, or pH shifts significantly

Limitations & Technical Considerations

- Requires a hydrocarbon supplement — the base broth alone will not support growth.
- Not a quantitative TPH/PAH assay by itself — quantifying degradation requires additional analytical methods (e.g., GC-FID, gravimetric analysis).
- 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.
- SIM Committee on Microbiological Deterioration of Fuels. Recommended procedures for the microbiological examination of fuels.
- American Public Health Association. Standard Methods for the Examination of Water and Wastewater (latest edition).

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