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TDS-AS-1447
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Jensen's Medium Broth (Nitrogen-Free)

Selective Liquid Medium for Free-Living Nitrogen-Fixing Bacteria | Catalog No. AS-1447

1. Product Identity

Product Name	Jensen's Medium Broth (Nitrogen-Free)
Catalog Number	AS-1447
Synonyms	Jensen's Broth, Jensen's Nitrogen-Free Broth, Azotobacter Enrichment Broth
Medium Type	Nitrogen-free, dehydrated liquid medium (no agar)
Weighed Quantity	24.1 g/L
Final pH (25°C)	7.2 – 7.8
Appearance (prepared)	Cream coloured, opalescent suspension with fine precipitate

2. Overview

Jensen's Medium Broth is the liquid form of Jensen's nitrogen-free formulation, used for enrichment, detection, and cultivation of free-living nitrogen-fixing bacteria, particularly Azotobacter species. Nitrogen-fixing organisms are free-living bacteria that grow well on a nitrogen-free medium, utilising atmospheric nitrogen gas for cell protein synthesis — cell protein that is subsequently mineralised in soil after cell death, contributing to nitrogen availability for crop plants.

As with the agar format (AS-1444), selectivity comes from what is absent: because no nitrogen source is added, only organisms capable of biological nitrogen fixation grow well, while non-fixing contaminants are suppressed by nitrogen limitation. The broth format is preferred for enrichment culture prior to plating, turbidity-based screening, and liquid-phase downstream work.

3. Historical Development

The formulation originates from Danish-Australian soil microbiologist H.L. Jensen's 1942 paper, “The Azotobacter of Danish agricultural soils,” published in the Proceedings of the Linnean Society of New South Wales (57, 205–212), part of a series of studies by Jensen through the early 1940s on nitrogen-fixing and cellulose-decomposing soil bacteria. The medium remains in active use in current research: a 2023 study used Jensen's nitrogen-free medium to culture reference strain Azotobacter vinelandii (ATCC BAA-1303) for label-free imaging of diazotroph activity, underscoring the formulation's continued relevance more than eight decades after its original description.

4. Principle & Mode of Action

Sucrose acts as the sole energy source, supporting the high metabolic cost of nitrogen fixation. Sodium molybdate supplies molybdenum, an essential cofactor of the nitrogenase enzyme complex; ferrous sulfate provides iron, also required for nitrogenase function. Dipotassium phosphate buffers the medium; sodium chloride maintains osmotic equilibrium; calcium carbonate stimulates growth and, in the broth format, settles as a fine suspension that should be resuspended by mixing before inoculation and periodically during incubation.

5. Broth vs. Agar — Format Comparison

Feature	AS-1447 (Broth)	AS-1444 (Agar)
Format	Liquid, no agar	Solid, 15 g/L agar
Total dissolution	24.1 g/L	39.1 g/L
Best for	Enrichment culture, turbidity screening, liquid-phase downstream work	Colony isolation, morphology observation, single-colony purification
Typical workflow	Enrich in broth first, then streak to agar for isolated colonies	Direct plating from soil dilutions for colony counts and isolation

6. Typical Applications

- Enrichment of Azotobacter spp. from soil and rhizosphere samples (liquid enrichment increases recovery of low-abundance diazotrophs prior to plating)
- Agricultural microbiology research — biological nitrogen fixation and soil nitrogen cycling
- Biofertiliser development — liquid-scale screening and propagation of candidate nitrogen-fixing strains
- Reference-strain maintenance — culturing diazotroph reference strains for downstream imaging, physiology, or genetic studies

7. Composition (per Litre)

Ingredient	Quantity	Function
Sucrose	20.000 g	Energy source
Calcium carbonate	2.000 g	Growth stimulant
Dipotassium phosphate	1.000 g	Buffering, phosphorus source
Magnesium sulfate	0.500 g	Mg^{2+} ion source
Sodium chloride	0.500 g	Osmotic equilibrium
Ferrous sulfate	0.100 g	Nitrogenase cofactor

Ingredient	Quantity	Function
Sodium molybdate	0.005 g	Nitrogenase cofactor

Total: 24.1 g/L (no agar). Due to calcium carbonate, the prepared broth forms a characteristic opalescent suspension with fine precipitate — this is expected and should be resuspended by gentle mixing before inoculation.

8. Preparation

- Suspend 24.1 g in 1 litre of distilled/purified water. Heat just to boiling to dissolve
- Dispense into flasks or tubes at the required volume before sterilisation
- Sterilise by autoclaving at 121°C for 15 minutes
- Mix well to resuspend the calcium carbonate precipitate before inoculation, and periodically during incubation to keep the suspension homogeneous

9. Quality Control (Growth Promotion Testing)

Test Organism	Inoculum	Expected Result (48–72 h, 28–30°C)
Azotobacter chroococcum (reference strain)	≤ 100 CFU	Good growth (visible turbidity) without added nitrogen source
Azotobacter vinelandii ATCC BAA-1303	≤ 100 CFU	Good growth (visible turbidity); reference strain used in current diazotroph research
Escherichia coli ATCC 25922	≤ 100 CFU	No growth to markedly inhibited growth (selectivity check — non-fixing organism)

10. Storage & Stability

- Dehydrated powder: store below 30°C, tightly closed, protected from moisture and light. Hygroscopic
- Prepared broth: store at 2–8°C. Discard if contaminated or discoloured. Use freshly prepared medium where possible

11. Intended Use

For laboratory and agricultural research use only. Not intended for therapeutic, diagnostic, or food-safety testing applications.

12. Literature / References

- Jensen, H.L. (1942). The Azotobacter of Danish agricultural soils. Proceedings of the Linnean Society of New South Wales, 57, 205–212.

- Subba Rao, N.S. (1977). Soil Microorganisms and Plant Growth. Oxford and IBH Publishing Co., New Delhi.
- Label-free functional analysis of root-associated microbes with dynamic quantitative oblique back-illumination microscopy. PMC10635382 (2023) — Jensen's nitrogen-free medium used with Azotobacter vinelandii ATCC BAA-1303.