

Jensen's Medium (Nitrogen-Free)

Selective medium for detection and cultivation of free-living nitrogen-fixing bacteria.

OVERVIEW

Jensen's Medium, formulated according to Jensen, is recommended for the 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 their cell protein synthesis. This cell protein is subsequently mineralised in soil after cell death, contributing to the nitrogen availability of crop plants. (Note: free-living nitrogen fixers such as *Azotobacter* differ from symbiotic nitrogen fixers such as *Rhizobium*, which enter into symbiosis with leguminous plant roots to form nodules.)

Because no nitrogen source is added, this medium is inherently selective: only organisms capable of biological nitrogen fixation can grow well, while non-fixing contaminants are suppressed by nitrogen limitation.

PRODUCT IDENTIFICATION

Product Name	Jensen's Medium (Nitrogen-Free)
Catalogue Number	AS-1444
Medium Type	Selective solid medium — free-living nitrogen-fixing bacteria
Reference Formula	Jensen, H.L.
Dissolution	39.1 g/L in distilled/purified water
Final pH (25°C)	7.2 – 7.8
Sterilisation	Autoclave 121°C for 15 minutes
Prepared Medium Appearance	Cream coloured, slightly opalescent gel with slight precipitate
Dehydrated Appearance	Off-white, fine, free-flowing, hygroscopic powder

MODE OF ACTION

Sucrose acts as the sole energy source, supporting the high metabolic cost of biological nitrogen fixation. No nitrogen compound is added to the formula, so growth selects for organisms able to fix atmospheric nitrogen (N_2) via the nitrogenase enzyme complex. Sodium molybdate supplies molybdenum, an essential cofactor of nitrogenase, directly supporting and enhancing nitrogen fixation activity. Ferrous sulfate provides iron, also required for nitrogenase function. Dipotassium phosphate buffers the medium and supplies phosphorus; sodium chloride maintains osmotic equilibrium; calcium carbonate stimulates growth and is a characteristic component of nitrogen-fixing bacterial isolation media, contributing to the medium's typical opalescent appearance with a fine precipitate.

FORMULA — PER LITRE

Component	g/L	Role
Sucrose	20.000	Energy source — supports high metabolic demand of N_2 fixation
Calcium Carbonate	2.000	Growth stimulant; contributes to characteristic opalescence
Dipotassium Phosphate (K_2HPO_4)	1.000	Buffering agent, phosphorus source
Magnesium Sulfate	0.500	Mg^{2+} ion source, enzyme cofactor

Sodium Chloride	0.500	Osmotic equilibrium
Ferrous Sulfate	0.100	Iron source — nitrogenase cofactor
Sodium Molybdate	0.005	Molybdenum — essential nitrogenase cofactor
Agar	15.000	Solidifying agent

Total: 39.1 g/L. Final pH 7.2–7.8 at 25°C. Note: due to the presence of calcium carbonate, the prepared medium forms a characteristic opalescent solution with a slight white precipitate — this is expected and not a quality defect.

PREPARATION PROTOCOL

Step	Instruction
1	Suspend 39.1 g of dehydrated Jensen's Medium (AS-1444) in 1 litre of distilled or purified water.
2	Heat just to boiling to dissolve the medium completely.
3	Sterilise by autoclaving at 121°C for 15 minutes.
4	Cool to 45–50°C, mix well (to resuspend the calcium carbonate precipitate), and pour into sterile Petri plates.
5	Allow to solidify before inoculation.

INCUBATION PARAMETERS

Purpose	Temperature	Duration	Expected Result
Azotobacter and free-living N-fixer screening	28–30°C	3–7 days	Mucoid, often pigmented colonies; good growth without added nitrogen

APPLICATIONS

Application	Notes
Isolation and screening of Azotobacter spp.	Standard method for detecting free-living nitrogen-fixing bacteria from soil and rhizosphere samples
Agricultural microbiology research	Study of biological nitrogen fixation and soil nitrogen cycling
Biofertiliser development	Pre-screening of candidate nitrogen-fixing strains for biofertiliser formulation

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

STORAGE & STABILITY

Dehydrated Powder	Store below 30°C in a tightly closed container, protected from moisture and direct sunlight. Hygroscopic — reseal promptly after use.
Prepared Medium	2–8°C. Discard if evidence of contamination, discolouration, or drying is observed.
Shelf Life	Refer to expiry date on label.

LITERATURE REFERENCES

- Jensen HL. Nitrogen-free medium for the isolation and cultivation of free-living nitrogen-fixing bacteria (Azotobacter spp.). Formulation attributed to H.L. Jensen and widely reproduced in standard microbiology media manuals.
- Vincent JM. A Manual for the Practical Study of Root-Nodule Bacteria. IBP Handbook No. 15. Blackwell Scientific Publications; 1970. [General reference for nitrogen-fixing bacteria isolation methodology]
- Atlas RM. Handbook of Microbiological Media, 4th ed. CRC Press; 2010. [Jensen's Medium formulation and application]

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