

TECHNICAL DATA SHEET

Ashby's Mannitol Agar

Principle

Ashby's mannitol agar is described by Subba Rao (1977) for isolation of a non-symbiotic nitrogen fixing bacteria, *Azotobacter*. Ashby's media composed of mannitol, dipotassium phosphate, magnesium sulphate, sodium chloride, potassium sulphate, calcium carbonate. Mannitol provides carbon source and atmospheric nitrogen serves nitrogen source. Dipotassium phosphate is buffering agent. The other salts provide various essential ions required for promoting growth of *Azotobacter*. The presence of calcium carbonate stimulate growth and increase availability of phosphorus, sulphur and magnesium to the organisms.

Use: For isolation of *Azotobacter* species from soil that can use mannitol and atmospheric nitrogen as source of carbon and nitrogen respectively.

Contents*

Ingredients	Gram/Liter
Mannitol	20.00
Dipotassium Phosphate	0.20
Magnesium Sulphate	0.20
Sodium Chloride	0.20
Potassium Sulphate	0.10
Calcium Carbonate	5.00
Agar	15.00
pH at 25°C	7.4 ±0.2

* Formula adjusted for optimum performance and parameters

Directions: Dissolve 40.70 grams in 1000 ml distilled water. Sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 min, cool it to 42-45 °C and distribute in desire. Ensure complete solidification and inoculate test sample aseptically.

Specimens types analyzed

Soil and water samples etc.

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Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Off white colored free flowing, homogeneous powder
Reaction of 4.0% solution	7.4±0.2 at 25 °C
pH	7.20- 7.60
Gelling	Firm comparable with 1.5% agar gel
Color and clarity of ready medium	Milky white colored opalescent gel with slight precipitate.
Growth Promotion properties	Best at ≤ 100 CFU at 32-37 °C for 3-5 days
Indicative properties	Optimum at ≤ 100 CFU at 32-37 °C for 3-5 days
Negative control	Performed using sterile distilled water

Different Microbial Response: Cultural characteristics observed after incubation at 33-37°C for 5 days. Inoculum 50-100 CFU.

Organism	ATCC	Growth
<i>Azotobacter nigricans</i>	35009	Luxuriant
<i>Azotobacter vinelandii</i>	478	Luxuriant

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

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Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. *Subba Rao, (1977), Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co., India.*

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