



Azotobacter Agar (Mannitol)

Intended Use:

Recommended for isolation, cultivation and identification of mannitol positive Azotobacter species from soil.

Composition**

Ingredients	Gms / Litre
Dipotassium hydrogen phosphate	1.000
Magnesium sulphate	0.200
Sodium chloride	0.200
Ferrous sulphate	TRACE
Soil extract	5.000
Mannitol	20.000
Agar	15.000
Final pH (at 25°C)	8.3±0.2
**Formula adjusted, standardized to suit performance parameters	

Directions

Suspend 41.4 grams in 1000 ml of purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If slight precipitate occurs after autoclaving, distribute it evenly before pouring into sterile Petri plates.

Principle And Interpretation

Bacteria of the family *Azotobacteraceae* constitute the majority of heterotrophic free-living nitrogen fixing bacteria (5). *Azotobacter* is a genus of free-living diazotrophic bacteria which have the highest metabolic rate compared to any other

microorganisms. *Azotobacters* have generated a good deal of interest in the scientific community because of their unique mode of metabolism, by which they can fix nitrogen aerobically. Azotobacter Agar (Mannitol) is used for isolation and cultivation of mannitol positive *Azotobacter* species from soil (4). It is also useful for maintenance of *Azotobacter* species by adding extra 1% Mannitol to the medium as specified by the American Type Culture Collection (1).

Type of specimen

Soil samples

Specimen Collection and Handling

For soil samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Some species may show poor growth due to nutritional variations.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Off white to beige homogeneous free flowing powder

Gelling Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured, clear to slightly opalescent gel with a slight precipitate forms in Petri plates.

Reaction

Reaction of 4.14% w/v aqueous solution at 25°C. pH : 8.3±0.2

pН

8.10-8.50

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 24-48 hours.

Organism	Growth

Azotobacter beijerinckii good-luxuriant *ATCC 12981 Azotobacter nigricans ATCC* good-luxuriant *35009*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

- 1. ATCC Catalogue of Bacteria and Bacteriophages, 1992, 18th Ed, American Type Culture Collection, Rockville, MD.
- 2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
- Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
- 4. Pelczar M. Jr., 1957, Manual of Microbiological Methods.
- 5. Subba Rao N. S., 1977, Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co., New Delhi.

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