Ashby's Mannitol Broth

Intended Use
Ashby's Mannitol Broth is used for isolation of *Azotobacter* species from soil.

Summary
Azotobacter is a genus of free-living diazotrophic bacteria which have the highest metabolic. *Azotobacters* are chemoorganotrophic, using sugars, alcohols and salts of organic acids for growth. They can non-symbiotically fix atmospheric nitrogen aerobically by unique mode of metabolism. They also synthesize biologically active substances, which attributes to improving seed germination, plant growth etc.

Principle
Ashby's Media are formulated as described by Subba Rao, 1977. It is used for isolation of *Azotobacter*, a non-symbiotic nitrogen fixing bacteria which uses mannitol as a carbon source and atmospheric nitrogen as nitrogen source. Dipotassium phosphate provides buffering to the medium. Various essential ions required for promoting growth of *Azotobacter* are also available in this medium.

Formula*

Ingredients	**g/L**
Mannitol	20.0
Dipotassium Phosphate	0.2
Magnesium Sulphate	0.2
Sodium Chloride	0.2
Potassium Sulphate	0.1
Calcium Carbonate	5.0
Final pH (at 25°C)	7.4± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Soil and Water samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend the 25.70 g of the powder in 1000 mL purified / distilled water.
2. Mix thoroughly.
3. Heat gently with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Quality Control
Dehydrated Appearance: White coloured, homogenous, free flowing powder.
Prepared Appearance: White coloured, opalescent solution with heavy white precipitate on standing the precipitate settles down.

Cultural Response: Cultural Response is observed after an incubation at 30°C-35°C for ≤ 5 days. Subculturing is carried out using Ashby's Mannitol Agar after enrichment in Ashby's Mannitol Broth at 30°C-35°C for ≤ 5 days.

Organism (ATCC)	**Growth**
Azotobacter nigricans (35009)	Good
Azotobacter vinelandii (478)	Good

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Subba Rao, 1977, Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co., India.
2. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	**Product description**	**Pack Size**
201010310100	Dehydrated Culture Media	100 g
201010310500	Dehydrated Culture Media	100 g