Azotobacter Broth (Mannitol) is recommended for cultivation of mannitol positive Azotobacter species from soil.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipotassium phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.200</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>TRACE</td>
</tr>
<tr>
<td>Soil extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Mannitol</td>
<td>20.000</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C)** 8.3±0.2

**Formula adjusted, standardized to suit performance parameters**

**Directions**
Suspend 26.4 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Slight precipitate may occur after autoclaving, however it will not interfere with growth performance nor interfere with the interpretation of results.

**Principle And Interpretation**

Azotobacter is a free-living nitrogen-fixing bacterium (3), which is used as a biofertilizer in the cultivation of most crops. Azotobacter is found on neutral to alkaline soils, in aquatic environments, in the plant rhizosphere and phyllosphere. Azotobacter species are gram-negative aerobic soil-dwelling bacteria and are usually motile, oval, or spherical bacteria, form thick-walled cysts, and may produce large quantities of capsular slime. They are typically polymorphic, i.e. of different sizes and shapes. Their size of the cells ranges from 2-10 µm long and 1-2 µm wide. Plant needs nitrogen for its growth and Azotobacter fixes atmospheric nitrogen non-symbiotically. Therefore, all plants, trees, vegetables, get benefited. Beyond Azotobacter is used as a model it has biotechnological applications like use for alginate production and for nitrogen production in batch fermentations. This medium contains necessary nutrients for growth of Azotobacter species. For cultivation of mannitol positive Azotobacter species from soil, Azotobacter broth (Mannitol) can be used (1). It is used for cultivation of mannitol positive Azotobacter species from soil. It can also be useful for maintenance of Azotobacter species by adding extra 1% Mannitol to the medium containing agar i.e solid media as specified by the American Type Culture Collection (2).

**Quality Control**

**Appearance**
White to Cream homogeneous free flowing powder

**Colour and Clarity of Prepared medium**
Colourless to off-white coloured clear to slightly opalescent solution with slight precipitate forms intubes

**Reaction**
Reaction of 2.64% w/v aqueous solution at 25°C. pH : 8.3±0.2

**pH**
8.10-8.50

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azotobacter beijerinckii ATCC 12981</td>
<td>50-100</td>
<td>luxuriant</td>
</tr>
</tbody>
</table>
Azotobacter nigricans ATCC 50-100 luxuriant 35009

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
2. ATCC Catalogue of Bacteria and Bacteriophages, 1992, 18th ed, American Type Culture Collection, Rockville, MD.

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