**Intended Use**
Recommended for cultivation of *Azospirillum* species.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Part A:</strong></td>
<td></td>
</tr>
<tr>
<td>Malic acid</td>
<td>5.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>0.500</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>0.500</td>
</tr>
<tr>
<td>Manganese sulphate</td>
<td>0.010</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.200</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.100</td>
</tr>
<tr>
<td>Bromo thymol blue</td>
<td>0.002</td>
</tr>
<tr>
<td>Sodium molybdate</td>
<td>0.002</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>Part B:</strong></td>
<td></td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>4.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>6.8±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 6.33 grams in 950 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and slowly add 50 ml of sterile Part B (prepared by adding 1.30 grams -1.50 grams of Potassium hydroxide to 50 ml of sterile purified / distilled water). Mix well and dispense into sterile tubes or flasks as desired.

**Principle And Interpretation**

*Azospirilla* are generally gram-negative rods which are motile by means of a single flagellum. *Azospirilla* are also capable of fixing nitrogen (1). The cells of *Azospirillum* remain in association with the roots and fix part of the atmospheric nitrogen. *Azospirillum* species may be isolated from rhizosphere biofilms associated with the roots of various grasses, cereals, and tuber plants. Their isolation is based on the fact that these organisms can grow in concentrations of nitrogen too low to support growth of most microorganisms. *Azospirillum* cultures are useful for the cereals and cash crops viz. wheat, paddy, bajra, jowar, maize, mustard, cotton, cumin, banana, sugarcane, tobacco, castor, vegetables etc., as well as horticultural crops. Both in greenhouse and in field trials, several strains of *Azospirillum* have been shown to exert beneficial effects on plant growth and crop yields, under various soil and climatic conditions, and are thus qualified as Plant Growth-Promoting Rhizobacteria (PGPR) (2).

This medium contains malic acid which serves as the prime carbon source. *Azospirillum* species grow well in presence of malic acid and are not overgrown by other nitrogen fixers. Dipotassium phosphate provides buffering effect. Necessary trace elements like ferrous sulphate, manganese sulphate, sodium molybdate supports growth of *Azospirillum* species. Divalent salts like calcium and magnesium helps metabolism of cells. Sodium chloride helps to maintain osmotic balance of cell.

**Type of specimen**
Soil sample

**Specimen Collection and Handling**
For soil samples, follow appropriate techniques for sample collection and processing as per guidelines (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. Due to variable nutritional requirements, some strains show poor growth on this medium.

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
Part A : Cream to yellow homogeneous free flowing powder Part B : White to cream pellets

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent solution

Reaction
Reaction of 0.63% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH
6.60-7.00

Cultural Response
Cultural characteristics observed after an incubation at 25-30°C for upto 8 days.

Organism Growth
Azospirillium brasilense luxuriant
ATCC 29710

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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. Use before expiry date on the label.

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 (3,4).

Reference