

Technical Information

Rhizobium Medium

Product Code: DM 1408

Application: Rhizobium Medium is used for cultivation and isolation of *Rhizobium* species.

Composition**

| Ingredients | Gms / Litre |
|-----------------------|-------------|
| Mannitol | 10.000 |
| Dipotassium phosphate | 0.500 |
| Magnesium sulphate | 0.200 |
| Yeast extract | 1.000 |
| Sodium chloride | 0.100 |
| Agar | 20.000 |
| Final pH (at 25°C) | 6.8±0.2 |

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Rhizobium Medium is recommended for isolation and cultivation of mannitol-positive *Rhizobium* species. By adding extra 1% mannitol to the medium as specified by the American Type Culture Collection ⁽¹⁾ Rhizobium species can be maintained for a larger period. Rhizobium Medium is used in the large scale production of legumes and in their isolation from root nodules.

The medium is well buffered for pH changes and osmotic changes by presence of phosphate and sodium chloride salts. Yeast extract provides nitrogenous nutrients. Mannitol is the energy source while magnesium sulphate provides essential ions.

The inocula are transferred from agar slants into starter flasks containing Rhizobium Medium. After 4 days of growth, the culture from starter flasks is transferred into a small seed tank fermentor. At this stage, Rhizobium Medium is used for large scale production. *Rhizobium* may be isolated from the root system of the leguminous plant. The healthy, pinkish nodule on the tap root is carefully cut out. The nodule is surface sterilized for 5 minutes and then washed with solvents like ethanol etc. The nodule is then crushed with a sterile glass rod in a small aliquot of sterile water. Serial dilutions are subsequently made to get sparse and distinct colonies. The dilutions are plated on Rhizobium Medium and incubated for upto 4 days at 25-30°C ⁽²⁾.

Methodology

Suspend 31.8 grams of powder media in 1000 ml distilled water. Shake well & heat to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Quality Control

Physical Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium

Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 3.18% w/v aqueous solution at 25°C. pH : 6.8±0.2



Dehydrated Culture Media
Bases / Media Supplements

pH Range:-
6.60-7.00

Cultural Response/Characteristics

DM 1408: Cultural characteristics observed after an incubation at 25-30°C for upto 4 days.

Organism

Growth

Rhizobium japonicum
ATCC 10324

luxuriant

Rhizobium meliloti
ATCC 9930

luxuriant

Storage and Shelf Lifez

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8⁰ in sealable plastic bags for 2-5 days.

Further Reading

1. ATCC Catalogue of Bacteria and Bacteriophages, 1992, 18th Ed., American Type Culture Collection, Rockville, MD.
2. Subba Rao N. S, Soil Microorganisms and Plant Growth- (Oxford and IBH Publishing Co.)

Disclaimer :

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