



Technical Data

Inorganic Salt Medium (Modified Raggios Medium)

M723

Intended Use:

Recommended for studying soil microorganisms such as *Rhizobium* species.

Composition**

Ingredients	Milligrams / Litre
Calcium carbonate	3000.000
Calcium chloride hexahydrate	446.000
Potassium chloride	165.000
Potassium dihydrogen phosphate	200.000
Magnesium sulphate heptahydrate	700.000
Sodium sulphate	200.000
Potassium iodide	0.750
Ferric chloride hexahydrate	2.500
Boric acid	1.500
Sodium molybdate dihydrate	0.250
Manganese sulphate tetrahydrate	6.640
Zinc sulphate heptahydrate	2.670
Copper sulphate pentahydrate	0.070

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 4.14 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat just to boiling. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired. *Note: Due to presence of calcium carbonate, the medium forms opalescent solution with white precipitate.*

Principle And Interpretation

Rhizobia are nitrogen-fixing bacteria, which live freely in soil and in the root region of both leguminous and non-leguminous plants. These bacteria are capable of forming complex symbiotic relationships with only leguminous plants. In an effective symbiosis, the bacteria infect the plant roots and induce the formation of specialized structures called nodules. In the nodules, the bacteria multiply, form specialized cells called bacteroids and subsequently convert atmospheric nitrogen to ammonia. Energy for the reduction of nitrogen is provided by the plant, through photosynthesis and the resultant product provides a nitrogen source for the plant (1). Inorganic Salt Medium (5) modified as per Bunting and Horrocks (2) is used for studying and isolation of soil microorganisms, such as *Rhizobium* species. Calcium stimulates nodulation when present as chloride or sulphate. Molybdenum is essential for symbiotic nitrogen fixation and stimulates the nitrogen fixing activity of the nodular tissue. Inorganic Salt Medium is used to moisten the sand (or used as such) into which the suspended roots grow.

Type of specimen

Soil sample- root system of the leguminous plant

Specimen Collection and Handling:

For soil samples follow appropriate techniques for handling specimens as per established 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. Individual organisms differ in their growth requirement and may show variable growth patterns on the 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

White to light yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Light yellow clear solution with heavy white precipitate at bottom.

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for upto 7 days.

Organism	Growth
<i>Rhizobium leguminosarum</i> ATCC 10004	luxuriant
<i>Rhizobium phaseoli</i> ATCC 14482	luxuriant
<i>Rhizobium trifolii</i> ATCC 14480	luxuriant

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 (3,4).

Reference

1. Balows A., Truper H. G., Dworkin M., Harder W., Schleifer K. H., (Eds.), The Prokaryotes, 2nd Edition, Vol. III, Springer-Verlag.
2. Bunting A. H. and Horrocks J., 1964, Ann. Bot., 28:229.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. Subba Rao N. S., 1977, Soil Microorganisms and Plant Growth, Oxford and IBH publishing Co., New Delhi.

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