Azospirillum Medium w/ 0.17% Agar (Twin Pack)

M518

Intended use

Azospirillum Medium with 0.17% Agar is used for the cultivation of *Azospirillum* species.

Composition**

Ingredients	Gms / Litre
Part A	-
Malic acid	5.000
Dipotassium hydrogen phosphate	0.500
Ferrous sulphate	0.500
Manganese sulphate	0.010
Magnesium sulphate	0.200
Sodium chloride	0.100
Bromo thymol blue	0.002
Sodium molybdate	0.002
Calcium chloride	0.020
Agar	1.750
Part B	-
Potassium hydroxide	4.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 8.08 grams of dehydrated Part A in 950 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add required quantity of Potassium hydroxide (Part B) dissolved in 50 ml of sterile distilled water to obtain pH of 6.8±0.2

(*)- As per standard it is recommended to use 4.000 grams of Potassium hydroxide (Part B)

Principle And Interpretation

Azospirillum species occur as free-living in soil or in association with the roots of cereal crops, grasses and tuber plants (1). *Azospirillum* species are plant-associated diazotrophs of the alpha subclass of *Proteobacteria*. Azospirillum Medium with 0.17% Agar is used for cultivation of *Azospirillum* species. Malic acid is used as the carbon source. *Azospirillum* species grow well in presence of Malic acid and are not overgrown by other nitrogen fixers. Dipotassium phosphate provides buffering effect and other inorganic salt ingredients provide necessary growth nutrients. Agar at 0.17% concentrations provides microaerophillic conditions necessary for nitrogen fixation by *Azospirillum* species (1).

Type of specimen

Soil samples.

Specimen Collection and Handling

For soil samples, follow appropriate techniques for sample collection, processing (1)
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. Further biochemical tests must be carried out for confirmation.

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

Gelling

Semisolid, comparable with 0.17 % Agar gel.

Colour and Clarity of prepared medium

Light yellow to pale green coloured clear to slightly opalescent solution.

Reaction

Reaction of 0.81% w/v aqueous solution (containing KOH) at 25°C pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

Cultural characteristics observed after an incubation at 30°C for upto 8 days

Organism	Growth
Azospirillium brasiliensis *ATCC 29710*	good-luxuriant

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. 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 (2,3).

Reference

1. Bergey's Manual of Determinative Bacteriology, 1994, 9th Ed, Williams R. H., (Eds.), Williams and Wilkins, Maryland, USA.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.

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