Hofer's Alkaline Medium

M717

Intended Use:

Recommended for selective isolation of *Agrobacterium* species while inhibiting *Rhizobium* species from soil samples.

Composition**

Ingredients	Gms / Litre
Mannitol	10.000
Dipotassium hydrogen phosphate	0.500
Magnesium sulphate	0.200
Sodium chloride	0.100
Yeast extract	1.000
Thymol blue	0.016
Agar	15.000
Final pH (at 25°C)	11.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.8 grams in 1000 ml purified/distilled water. Heat to boiling 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.

Principle And Interpretation

Agrobacterium is a genus of bacteria that causes tumours in plants. Most strains of *Agrobacterium* are plant pathogens and their natural habitat is on and around the roots and underground stems of susceptible plants (1). *Agrobacterium tumefaciens* is the most commonly studied species in this genus. *Agrobacterium* is well known for its ability to transfer DNA between itself and plants, and for this reason it has become an important tool for plant improvement by genetic engineering. Hofers Alkaline Medium is formulated as described by Subba Rao (4) and is used for growing *Agrobacterium* species while inhibiting *Rhizobium* species from soil. It is a selective medium with high alkaline pH. Agrobacteria grow at higher pH while Rhizobia fail to grow at alkaline pH. The medium is supplemented with mannitol as the carbohydrate or carbon source. Yeast extract provides nitrogenous nutrients.
Sodium chloride maintains osmotic balance of the medium. Dipotassium phosphate buffers the medium. Thymol blue is the pH indicator, which remains blue at high alkaline pH.

Type of specimen

Soil samples

Specimen Collection and Handling:

For soil samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(4) 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.
2. Further biochemical and serological tests must be carried out for further identification.

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

Light yellow to light green homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Blue coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.68% w/v aqueous solution at 25°C. pH : 11.0±0.2

pH
10.80-11.20

Cultural Response
Cultural characteristics observed after an incubation at 25-28°C for upto 5 days.

Organism	Growth
Agrobacterium luteum ATCC 25657	good-luxuriant
Agrobacterium tumefaciens ATCC 15955	good-luxuriant
Rhizobium trifolii ATCC 14480	inhibited

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 (2,3).

Reference

1. Balows A., Truper H. G., Dworkin M., Harder W., Scheifer K. H., (Eds.), The Prokayotes, 2nd Edition, Springer-Verlag, New York Inc.
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.
4. Subba Rao N. S., 1977, Soil Microorganisms and Plant Growth, Oxfordand IBH Publishing Co., New Delhi.

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