Citrate Agar

M728

Intended use

Recommended for cultivation of iron bacteria from soil samples.

Composition**

Ingredients	Gms / Litre
Ammonium sulphate	0.500
Sodium nitrate	0.500
Magnesium sulphate	0.500
Dipotassium phosphate	0.500
Calcium chloride	0.200
Ferric ammonium citrate	10.000
Agar	15.000
Final pH (at 25°C)	6.7±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.2 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. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

The iron bacteria oxidize ferrous iron to ferric state, which precipitate as ferric hydroxide around cells. These bacteria are usually non-filamentous and spherical or rod shaped. Certain algae also transform ferrous salts to ferric state and deposit the precipitation around the colonies. The ferric hydroxide deposits give a brown or rust red colour to these organisms. Citrate Agar is recommended by Subba Rao (5) for the isolation and detection of iron bacteria. A modification of the original formulation of Subba Rao is recommended by APHA (1) for the isolation of heterotrophic iron-precipitating bacteria (2). Dipotassium phosphate provides buffering to the medium. Magnesium sulphate, ammonium sulphate and calcium chloride are sources of ions that stimulate metabolism. Ferric ammonium citrate is used as a source of carbon and sodium nitrate acts as a source of nitrogen.

Type of specimen

Soil samples; Water samples

Specimen Collection and Handling

For soil samples, follow appropriate techniques for sample collection and processing as per guidelines (5).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(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. This medium is general purpose medium for soil bacteria and may not support the growth of fastidious organisms.

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
Cream to greenish yellow homogeneous free flowing powder

Gelling
Firm,comparable with 1.5% Agar gel

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

Reaction
Reaction of 2.72% w/v aqueous solution at 25°C. pH : 6.7±0.1

pH
6.60-6.80

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for upto 7 days.

Organism	Growth
Escherichia coli ATCC 25922 (00013*)	inhibited
Sphaerotilus natans ATCC 13338	good-luxuriant

Key :- Corresponding WDCM numbers

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. 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

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Clark F. M., Scott R. M. and Bone E., 1967, Heterotrophic, iron-precipitating bacteria, J. Am. Water Works Assoc., 59: 1036.
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, Oxfordand IBH Publishing Co., New Delhi.

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