

TECHNICAL DATA SHEET

Citrate Agar

Principle

Citrate Agar is recommended by Subba Rao (1977) for the isolation and detection of iron bacteria. A modification of the original formulation of Subba Rao is recommended by APHA (2015) for the isolation of heterotrophic iron-precipitating bacteria. The media composed of ferric ammonium citrate, sodium nitrate, magnesium sulphate, dipotassium phosphate, calcium chloride and agar. 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. The iron bacteria oxidize ferrous iron to ferric state, which precipitate as ferric hydroxide around cells. Certain bacteria also transform ferrous salts to ferric state and deposit the precipitation around the colonies. The ferric hydroxide deposits give a brown or rust red color to these organisms.

Use: For cultivation of iron bacteria from soil.

Contents*

Ingredients	Gram/Litre
Ammonium sulphate	0.500
Ferric ammonium citrate	10.000
Sodium nitrate	0.500
Magnesium Sulphate	0.500
Dipotassium Phosphate	0.500
Calcium Chloride	0.200
Agar	15.000
pH at 25°C	6.7 ±0.2

* Formula adjusted for optimum performance and parameters

Directions: Dissolve 27.20 grams in 1000 ml distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 min, cool it to 42-45 °C and distribute aseptically in desired. Ensure complete solidification and inoculate test sample aseptically.

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Specimens types analyzed Soil samples and biofertilizers etc.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Cream to greenish yellow colored free flowing, homogeneous powder
Reaction of 2.72% solution	6.7 ±0.2 at 25 °C
pH	6.50- 6.90
Gelling	Firm comparable with 1.5% agar gel
Color and clarity of ready medium	Light amber colored, slightly opalescent gel
Growth Promotion properties	Best at ≤ 100 CFU at 32-37 °C for 18-72 h
Indicative properties	Optimum at ≤ 100 CFU at 32-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

Different Microbial Response

Organism	ATCC	Inoculum	Growth
<i>Escherichia coli</i>	25922	50-100	inhibited
<i>Sphaerotilus natans</i>	13338	50-100	Good

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Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store in cool (10- 30°C) & Dry place (RH: < 40%). The declared expiry is valid only when stored as per the mentioned conditions.

Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Atlas, R. M. (2005). *Handbook of media for environmental microbiology*. CRC press.
2. 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.
3. Clark F. M., Scott R. M. and Bone E., (1967), *Heterotrophic, iron-precipitating bacteria*, *Journal American Water Works Association*, 59: 1036.
4. Subba Rao N. S., (1977), *Soil Microorganisms and Plant Growth*, Oxford and IBH Publishing Co., New Delhi.

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