

TECHNICAL DATA SHEET

BAT Agar (Alicyclobacillus Agar)

Principle

BAT agar is used for the enumeration, detection, and identification of spore-forming thermo-acidophilic spoilage bacteria, specifically *Alicyclobacillus* species. The media is composed of yeast extract, glucose, calcium chloride, magnesium sulfate, ammonium sulfate, potassium dihydrogen phosphate, and agar. Yeast extract is a source of nitrogenous compounds, vitamin B complexes, and other growth nutrients. Glucose as a carbon and energy source. Trace elements calcium chloride, magnesium sulfate, and ammonium sulfate supply the specific requirements of the spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* species). Potassium dihydrogen phosphate acts as a buffering system, and agar is a solidifying agent. The low pH (adjusted with 1N H₂SO₄) value and the high incubation temperature inhibit the contaminating flora.

Use: For the isolation of *Alicyclobacillus* species in fruit juices.

Contents*

Ingredients	Gram/Litre
Yeast Extract	2.000
Glucose	5.000
Calcium chloride dihydrate	0.250
Magnesium sulfate heptahydrate	0.500
Ammonium sulfate	0.200
Potassium dihydrogen phosphate	3.000
Agar	18.000
pH at 25°C	4.0 ± 0.2

*** Formula adjusted for optimum performance and parameters**

Directions: Dissolve 29.00 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 adjust the pH to 4.0 ± 0.2 with sterile 1N H₂SO₄. Mix well and pour into sterile petri plates. Avoiding air bubbles while pouring. Ensure complete solidification and inoculate test sample aseptically.

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ISO 9001-2008 Certified Company

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Specimens types analyzed

Fruit juice, fruit juice products, ingredients, environmental samples such as process water, beverages etc. This culture medium complies with the specifications given by IFU Method No. 12.

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	Beige colored free flowing, homogeneous powder
Reaction of 2.9% solution	4.0 ± 0.2 at 25 °C (Adjust pH after autoclave using 1N H ₂ SO ₄)
pH	3.80 – 4.20
Gelling	Firm comparable with 1.8% agar gel
Color and clarity of ready medium	Light amber color clear to slightly opalescent gel
Growth Promotion properties	Best at ≤ 100 CFU at 45 ± 2°C for 18-72 h
Indicative properties	Optimum at ≤ 100 CFU at 45 ± 2°C for 18-48 h
Negative control	Performed using sterile distilled water

Different Microbial Response: Cultural characteristics observed after an incubation at $45 \pm 2^{\circ}\text{C}$ for 48-72 hours. Inoculum 50-100 CFU.

Organism	ATCC	Growth	Recovery
<i>Alicyclobacillus acidoterrestris</i>	49025	Luxuriant	$\geq 70\%$
<i>Alicyclobacillus acidocaldarius</i>	27009	Luxuriant	$\geq 70\%$
<i>Escherichia coli</i>	8739	Inhibited	--

Storage and Shelf Life

The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label.

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. IFU International Fruit and Vegetable Juice Association. Method on the detection and enumeration of spore-forming thermo-acidophilic spoilage bacteria (*Alicyclobacillus* spp.). Method of Analysis No. 12:2019.
2. ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014+Amd1:2018.
3. Yokota, A., Fuji T., and Goto K. (eds) *Alicyclobacillus: Thermophilic Acidophilic Bacilli*. Springer, Japan, First Edition, 2007.
4. Witthuhn, R. C., W. Duvenage, and P. A. Gouws. "Evaluation of different growth media for the recovery of the species of *Alicyclobacillus*." *Letters in applied microbiology* 45.2 (2007): 224-229.

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