



BAT Agar

Instructions For Use

ENGLISH

Solid medium for detection and enumeration of *Alicyclobacillus* spp.

DESCRIPTION

BAT Agar is a medium used for the isolation and enumeration of *Alicyclobacillus* species in fruit juice and juice-related products [e.g. juice blends, juice concentrates (raw materials), carbonated fruit drinks and shelf stable ice tea)], and environmental samples, including process water and other samples from the juice processing plant.

This medium is formulated according to the specifications of IFU method No. 12: 2019 for detection of spoilage bacteria.

Alicyclobacillus spp. are spore-forming thermo-acidophilic bacteria which represent a real current threat to the beverage industry as they can contaminate the fruit juice production process at different points.

TYPICAL FORMULA*	(g/litre)
Yeast Extract	2.0
Glucose anhydrous	5.0
Calcium Chloride dihydrate	0.25066
Magnesium Sulfate heptahydrate	0.5
Ammonium Sulfate	0.2
Potassium Dihydrogen Phosphate	3.0
Zinc Sulfate monohydrate	0.00018
Copper Sulfate pentahydrate	0.00016
Manganese Sulfate hydrate	0.00015
Sodium Molybdate dihydrate	0.0003
Agar	20.0
Final pH 4.5 ± 0.2 at 25°C	

*Adjusted and/or supplemented as required to meet performance specifications.

METHOD PRINCIPLE

Yeast extract is source of vitamins, particularly of group B. Glucose is the fermentable carbohydrate providing carbon and energy. Calcium chloride and magnesium sulfate provide divalent cations. Ammonium sulfate provides sulfur. Potassium phosphate is the buffering agent. Zinc sulfate, copper sulfate, manganese sulphate and sodium molybdate provide trace elements which support growth of *Alicyclobacillus* species. The low pH of the medium along with an high incubation temperature inhibit the accompanying bacterial flora. Agar is the solidifying agent.

TEST PROCEDURE

Following the procedure in the IFU method No. 12, prepare the initial suspension by adding 10 g or 10 ml test portion to a 90 ml bottle of BAT Broth (ref. 402670) to yield a tenfold dilution. If necessary, prepare further dilutions (filterable sample and water samples need no dilution).

Heat treatment at 80 ± 1°C for 10 min.

For direct enumeration by filtration technique, cool the sample to 44-47°C in a water bath, mix well, and filter 100 ml through a membrane filter (0.45 µm pore size). Place the membrane onto a BAT Agar plate, ensuring that no air bubble is trapped between the filter and the medium surface.

Incubate the plates into a plastic bag at 45 ± 1°C for 5 days in ambient atmosphere with the membrane/agar surface uppermost.

For detection by enrichment, after the heat treatment incubate BAT broth aerobically at 45 ± 1°C for 5 days. Inoculate a BAT agar plate by spreading 0.1 ml of the enrichment broth onto the agar surface.

Incubate the plates in inverted position (bottom up) into a plastic bag at 45 ± 1°C for 48 ± 4 hours in ambient atmosphere. If negative, extend incubation for additional 72 ± 4 hours.

INTERPRETING RESULTS

Check the plating medium for the presence of colonies which are considered to be presumptive *Alicyclobacillus* spp.

Confirm selected colonies by streaking out onto one plate of BAT agar and one plate of a medium with neutral pH such as Plate Count Agar (ref. 10032).

Incubate both plates aerobically at $45 \pm 1^\circ\text{C}$ for 72 ± 4 h.

Regard as *Alicyclobacillus* those colonies which grow on the plate of BAT Agar but fail to grow on the other plate.

STORAGE

Store at $10\text{-}25^\circ\text{C}$ away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

Avoid quick temperature shifts to prevent condensation.

SHELF LIFE

6 months.

QUALITY CONTROL

Appearance: Slightly opalescent, amber.

Expected cultural response:

Control strain		Inoculum	Incubation	Specification
<i>Alicyclobacillus acidoterrestris</i>	DSM 2498	50-100 CFU	3-5 d / $45 \pm 1^\circ\text{C}$	Good growth ($P_R \geq 0.7$)
<i>Alicyclobacillus acidocaldarius</i>	DSM 446 (ATCC 27009)			
<i>Escherichia coli</i>	WDCM 00013 (ATCC 25922, NCTC 12241)	$10^4\text{-}10^6$ CFU		Total inhibition
<i>Bacillus subtilis</i>	WDCM 00003 (ATCC 6633 NCTC 10400)			

A productivity ratio (P_R) of 0.7 is equivalent to a recovery rate of 70%.

Please see the actual batch related Certificate of Analysis.

WARNING AND PRECAUTIONS

For professional use only. Operators must be trained and have certain experience. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

DISPOSAL OF WASTE

Disposal of waste must be carried out according to national and local regulations in force.

BIBLIOGRAPHY

See the references at the end of this document.

TABLE OF SYMBOLS

See the table of symbols at the end of this document.

The product is available in the configurations listed below. There may be additional product ref. numbers as well. For an updated listing of available products, visit liofilchem.com

Product	Format	Packaging	Ref.
BAT Agar	90 mm Plate	20 plates	10473

Revision History

Revision	Release Date	Change Summary
0	2021-11-22	Document creation
1	2023-10-18	Revised TYPICAL FORMULA











This IFU document and the SDS are available from the online Support Center:

liofilchem.com/ifu-sds

BIBLIOGRAPHY

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. EN ISO 11133:2014+Amd1:2018. Microbiology of food, animal feed and water -- Preparation, production, storage and performance testing of culture media.
3. Walker, M., Phillips, C.A. (2008). *Alicyclobacillus acidoterrestris*: an increasing threat to the fruit juice industry? International Journal of Food Science and Technology, 43, 250 – 260.
4. Chen, S., et al. (2006). Isolation and characterization of thermo-acidophilic endospore-forming bacteria from the concentrated apple juice-processing environment. Food Microbiology, 23, 439 – 445.
5. Matsubara, H. et al. (2002). *Alicyclobacillus acidiphilus* sp. Nov., a novel thermo-acidophilic, ω -alicyclic fatty acid-containing bacterium isolated from acidic beverages. International Journal of Systematic and Evolutionary Microbiology, 52, 1681 – 1685.

TABLE OF SYMBOLS

	Batch code
	Catalogue number
	Manufacturer
	Use by
	Fragile, handle with care
	Temperature limitation
	Contains sufficient for <n> tests
	Consult Instruction For Use
	Do not reuse
	Keep away from heat

**LIOFILCHEM® s.r.l.**Via Scozia, 64026 Roseto degli Abruzzi (TE) Italy
Tel. +39 0858930745 Fax +39 0858930330www.liofilchem.comliofilchem@liofilchem.com