



# User manual

**BAT-Agar (Product No. 2.04719.782)**

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## 1. Information

### Nutrient media for the detection of

*Alicyclobacillus* spp.

### in the beverage and fruit compound industry.

**BAT-Agar (pH 4.0 ± 0.2)** is an ready-to-use selective agar for isolation and microorganism count determination. In combination with the BAT-Broth and Guaiacol Detection Kit, it is a fast, reliable, sensitive and cost-efficient method for analysis of *Alicyclobacillus* spp. even in complex products and is compliant to the IFU Method "Method on Detection of taint producing *Alicyclobacillus* in Fruit Juices" (IFU Method No.12.)

Representatives of the genus of *Alicyclobacillus* spp. are large, immobile, spore-forming and thermophilic bacteria, which may spoil beverage and fruit juices by formation of an Off-flavour, which are repulsive changes in taste and odor. Its spores can survive normal pasteurisation treatments and may be activated and grow in favourable conditions after a few weeks. The product itself remains optically proper without gas formation or typical colour changes. Even small numbers of bacteria may lead to the spoilage of the product. Therefore in internal specifications of the beverage industry a zero tolerance is required.

Use the BAT-Agar as a basic culture medium for membrane filtration or pour plate and spread plate method.

The performance test of this culture medium is compliant with the current version of the EN ISO 11133.

## 2. Handling

### Required Material

Water bath (temp 95°C/203°F)

BAT-Broth (Product No. 2.04719.782) for cold filled ready-to-use drinks

Plate Count Agar, PCA (Product No. 8.76631.782)

Microbiological workbench

Petri dish

Sterile inoculation loop or Drigalski spatula

Incubator

### Application

Handle the samples under sterile conditions to avoid secondary contamination.

Melt the bottle content in a water bath at 95°C/203°F. Avoid overheating and remove the medium as soon as it is completely dissolved. The screw cap should be loosened before heating and closed tightly after removal from the water bath. To avoid glass breakage, it is recommended to leave the bottles on a heat-resistant surface at room temperature for a short period, e.g. 2 min, before placing them in a 45-50°C/113-122°F water bath to cool.

Ensure that the agar does not solidify again.



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Do not leave the liquefied agar for longer than 4 h at 45-50°C/113-122°F in a water bath. This damages the agar structure and permanently changes its solidifying properties. Repeated heating can restrain the functionality.

*For cold filled ready-to-use drinks and compounds:*

Spread 0,1 ml of the enriched bacterial culture (see BAT-Broth specifications) on the surface of BAT-Agar (pH 4.0) and Plate Count Agar (pH 7.0) using a sterile inoculation loop or a Drigalski spatula.

*For hot filled ready-to-use drinks:*

Incubate the product in the original package at 43-47°C/109-117°F for 7 days to obtain a bacterial enrichment.

Spread 0,1 ml of your enriched sample on the surface of BAT-Agar (pH 4.0) and Plate Count Agar (pH 7.0) using a sterile inoculation loop or a Drigalski spatula.

## **Incubation**

Incubate the inoculated plates (stored in a plastic bag to avoid drying) 3 days at a temperature of 43-47°C/109-117°F under aerobic conditions.

## **Evaluation**

Evaluate the samples macroscopically.

A lack of bacterial growth in form of colonies on the BAT-Agar indicates that the sample is *Alicyclobacillus*-negative.

A formation of bacterial colonies on BAT-Agar (pH 4.0) but not on Plate Count Agar (pH 7.0) indicates a positive result for *Alicyclobacillus spp.* (Fig.1).

A growth on Plate Count Agar (pH 7.0) indicates a negative result, as *Alicyclobacillus spp.* can only grow in an acidic environment.



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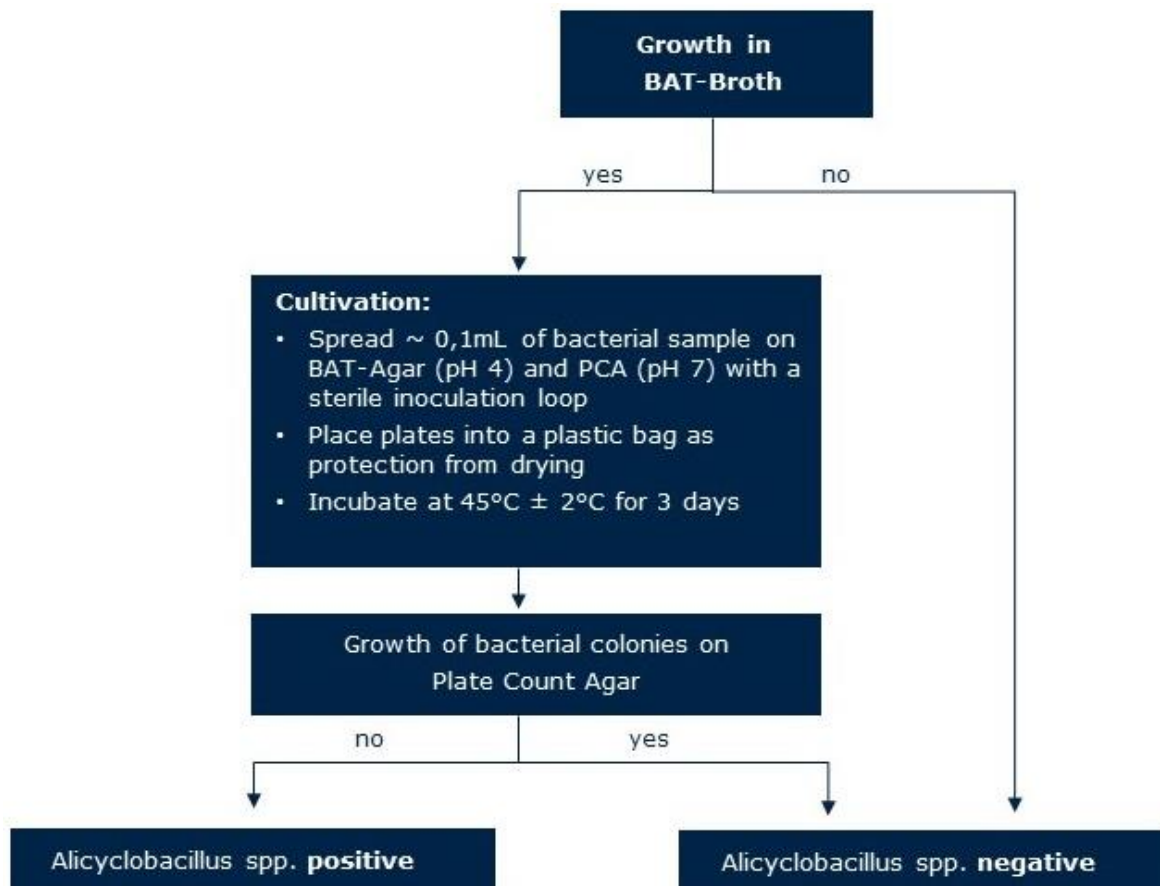


Fig. 1 Evaluation scheme for detection of *Alicyclobacillus* spp. in your sample

## 3. Storage and Packaging Information

### Packaging and Content

unit	Cardboard Box (9x250 ml in glass bottles)
unit size (Box)	approx. 22 cm x 22 cm x 18 cm / 8.7 in x 8.7 in x 7.1 in
unit gross weight (Box)	approx. 4.1 kg/ 9 lbs.

### Storage

Store at 4-8°C/40-46°F according to product specification.  
Store under dry and dark conditions. Do not freeze product.

### Waste Disposal

No dangerous good.



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No hazardous material.  
 Please consider your local waste regulations.  
 Not inoculated agar can be disposed of with normal laboratory waste.  
 Inoculated and incubated samples are to be sterilized before disposal at a temperature of 121°C/250°F for 20 min.

## Warnings

Do not cook or freeze the culture media. Do not keep the agar liquefied. Repeated heating can restrain its functionality. Always wear protective clothing when handling hot media.

## 4. Related Products

Investigation material	Relevant target microorganisms	Product	Format	Description	Packaging	pH (±0,2)	Incubation T [°C]	Item no.
<b>Beverage containing juice pH &lt; 4.5</b> Fruit juices Fruit juice concentrates Compounds Sugar	<i>Alicyclobacillus</i> spp.	<b>BAT-A</b>	Agar	Selective agar for isolation and microorganism count determination Application: Basic culture medium for membrane filtration, pour plate and spread plate method	9 x 250 mL (glass bottle)	4.0	43-47	2.04719.782
		<b>BAT-B</b>	Broth	Selective liquid medium for fast trace detection Application: Sample enrichment (especially of cloudy samples and beverage raw materials)	9 x 250 mL (glass bottle)	4.0	43-47	2.04717.782
		<b>Guaiaacol Detection Kit</b>	Detection kit	Enzymatic test kit for detection of off-flavour forming <i>Alicyclobacillus</i> spp.	Incl.40 tubes (box)		43-47	2.04737.991