User Manual

Guaiacol Detection Kit

Item No. 2.04737.991

The **Guaiacol Detection Kit** is a biochemical test for rapid and qualitative detection of guaiacol producing (off-flavour), acid-tolerant and heat resistant *Alicyclobacillus* spp.. Among the identified species so far, *A. acidoterrestris*, *A. acidiphilus* and *A. herbarius* show the potential for guaiacol formation which causes an off-flavour in fruit juices or fruit juice concentrates and compounds. This means that the detection of *Alicyclobacillus* bacteria in a product using classical culturing methods (e.g. BAT-Agar) does not allow a clear prediction on the extent and/or risk of beverage spoilage. The test kit allows identifying *Alicyclobacillus* spp. that pose a risk potential for beverage spoilage.
For this purpose, the *Alicyclobacillus* colonies previously isolated from the test sample on BAT-agar should be incubated in the ready-to-use incubation broth (IB). The biochemical test detects guaiacol by a visible color change in which colorless guaiacol is converted by an enzyme reaction to the brownish complex tetraguaiacol. Finally, the results for staining can be compared visually by running negative and positive samples in parallel. The test is part of IFU method no. 12 (2019) for the detection of guaiacol-forming *Alicyclobacillus* strains.

1. Media Preparation

The test kit contains ready-to-use media, chemical solutions and a vial of lyophilized enzyme. Before initial use, homogeneously dissolve the enzyme (solution 3) powder in 900 µL of sterile distilled water.

2. Application

Please work under sterile conditions to avoid secondary contamination of the samples.

Sample

- For each sample, label a supplied tube filled with BAT-IB
- Pick colonies to be tested with a sterile inoculation loop and suspend it in BAT-IB
- Incubate the tube for at least 3 h at 45±1°C
- Add 1 mL of buffer solution to all the tubes and mix well. Add 20 µL hydrogen peroxide solution (solution 2) and 20 µL enzymatic solution (solution 3) to the tubes.
- Mix well and incubate the samples for 5 - 10 minutes at room temperature. If guaiacol is present, a colour change from colourless to brown is seen after 10 minutes at the latest.

Negative control

- Treatment as indicated for sample analysis but without bacteria

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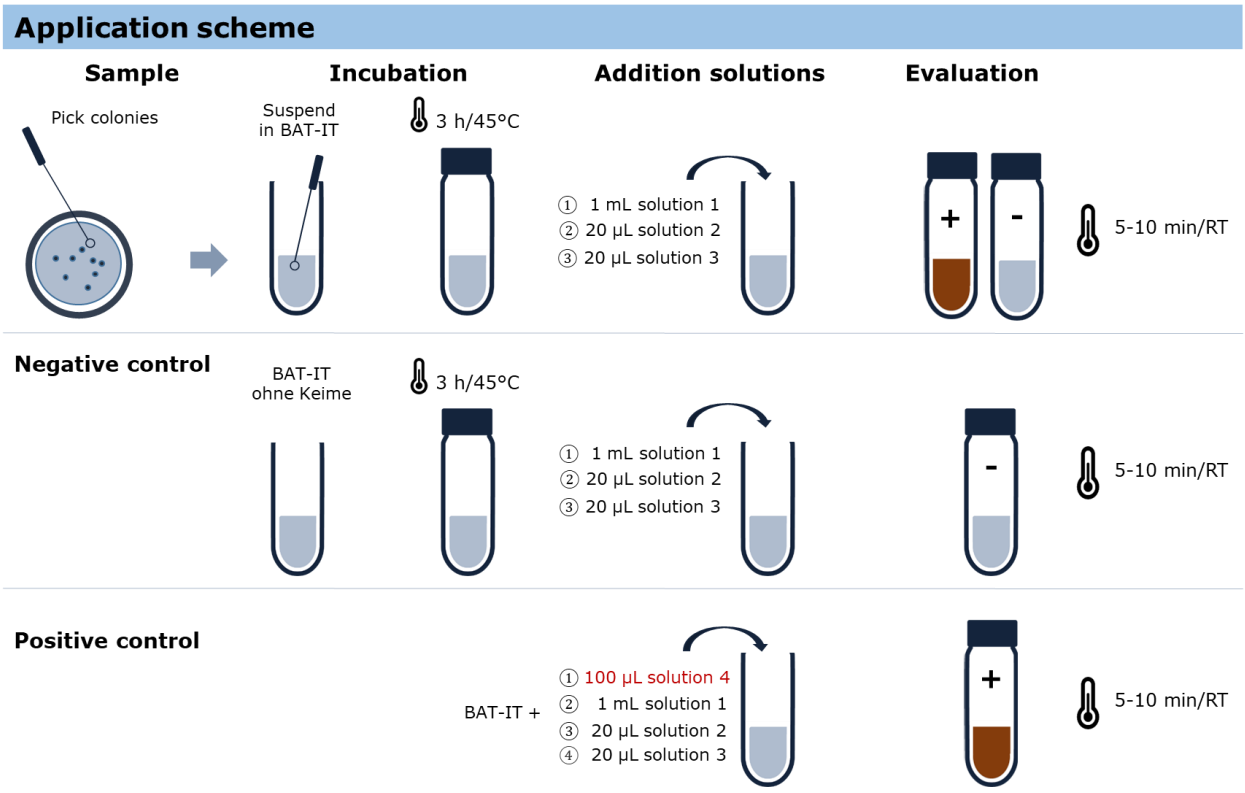
Positive control

- For the positive control, label a supplied tube filled with BAT-IB
- Pipette 100 µL standard solution (solution 4), 1 mL buffer solution (solution 1), 20 µL hydrogen peroxide (solution 2) and 20 µL enzyme solution (solution 3) into the positive control.
- Mix well and incubate the samples for 5 - 10 minutes at room temperature. A colour change from colourless to brown is visible after 10 minutes at the latest.



BAT-IB: BAT-Incubationbouillon, +: positive control, - : negative control

3. Evaluation and Result Interpretation

The presence of guaiacol is shown by the brown color. Beverage spoiling bacteria with a high potential of producing this off-flavor are present when the brown color of the sample is more intensive than the color of the negative control and a low potential is present if the color of sample and negative control are comparable. If color change is not clear, an increase of the incubation time up to 24 h is recommended. Guaiacol-free samples remain colorless.

4. Product Information

4.1 Packaging and content

Package/content: Guaiacol detection kit

- BAT-IB: 40 tubes filled with 2 mL incubation bouillon
- Solution 1: 50 mL buffer, pH 4.0
- Solution 2: 900 µL hydrogen peroxide solution
- Solution 3: lyophilized enzyme
- Solution 4 : 2x Guaiacol standard solution

Size: 30 cm x 25 cm x 20 cm

Weight gross: 1.8 kg

4.2 Storage and shelf life

Store in a dry and dark place at 4 - 8 °C. Shelf life of the test kit is 360 days (from production date). The ready-made enzyme solution should be used within 3 - 4 months. Do not freeze the reagents.

4.3 Waste disposal

- No dangerous goods and hazardous material
- Please consider your local waste regulations
- Non inoculated media can be disposed of with laboratory waste
- Inoculated and incubated media should be sterilized for 20 min at 121 °C before disposal

4.4 Warnings and precautions

It is recommended to wear protective clothing. Skin contact with reagents, especially with solution 2, should be avoided. Any contact with skin/eyes should be treated by washing/irrigation. This product is for use in microbiological control only and not intended for consumption or medical use. More information in SDS.

5. Quality Control

Guaiacol detection kit was tested for functionality with following microorganism.

Microorganism	Test result
Alicylobacillus acidoterrestris (DSM 3922)	Positive (Guaiacol forming)

6. Similar Products

Product	Item Nr.	Target microorganism
BAT-A	2.04719.782	*Alicyclobacillus* spp.
BAT-B	2.04717.782	*Alicyclobacillus* spp.
Plate Count Agar	8.76631.782	Gesamtkeimzahl

7. References

[1] IFU Method of Analysis No. 12 (2019): Method on the Detection and Enumeration of Spore-forming Thermo-Acidophilic Spoilage bacteria *(Alicyclobacillus* spp.)