



User manual

Guaiacol detection kit (Art.No. 2.04737.991)

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1. Information

Enzyme assay for the detection of *Guaiacol-producing Alicyclobacillus ssp.* in the beverage and fruit compound industry.

The Guaiacol detection kit is a reliable and sensitive enzymatic test kit for the detection of off-flavour forming *Alicyclobacillus* in fruit juices, fruit juice concentrates and compounds. The product is compliant to the Standard-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 a low contamination may lead to the spoilage of the product. Therefore in internal specifications of the beverage industry a zero tolerance is required.

Just the detection of *Alicyclobacillus* spp. only in a product does not allow a clear prediction on the extent and /or risk of beverage spoilage. From the *Alicyclobacillus* strains identified so far only *A. acidoterrestris*, *A. acidiphilus* und *A. herbarius* pose a risk in creating Off-flavours. As the classical culturing methods (e.g. BAT-Agar) only permit the identification of the entire group of *Alicyclobacillus* strains, the user needs to be able to distinguish the risk potential of the various *Alicyclobacillus* strains and clearly identify those with the greatest potential for beverage spoiling.

2. Handling

Required Material

Pipettes (1 mL, 100/200 µL)
Sterile pipette tips
Sterile inoculation loop

Application

Handle the samples under sterile conditions to avoid secondary contamination.

Store reagents (1-4) in a fridge (4-8°C/39-46°F) until usage.

Inoculate positive *Alicyclobacillus* sample from BAT-Agar into incubation tube with induction broth by using a sterile inoculation loop.

Prepare two additional incubation tubes without *Alicyclobacillus* sample for positive and negative control sample.

Incubate the tubes for min. 3 h at 45°C/113°F.

The enzyme powder, which is necessary for the colour reaction, has to be homogeneously solubilized in 900 µL sterile water before the first use.



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Successively add 1 mL buffer solution, 20 µL hydrogen peroxide solution and 20 µL enzymatic solution to all tubes including the positive and negatives control sample. Change pipette tips after every single pipette step in order to avoid secondary contamination.

Finally add 100 µL of Guaiacol standard solution (solution 4) to the positive control sample.

Evaluate the samples after 10 min. incubation at room temperature.

Table 1: Preparation of samples for the Guaiacol detection kit

Test Sample	Positive Control Sample	Negativ Control Sample
Suspension of bacterial colony in BAT-IB		
Incubation for at least 3 h at 45±1°C		
Add 1 mL solution 1 (buffer)		
Add 20 µL solution 2 (hydrogen peroxide)		
Add 20 µL solution 3 (enzyme solution)		
Mix tube and incubate 5-10 min at room temperature		
	Add 100 µL solution 4 (standard solution)	
Evaluation of color change after 10 min		



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Evaluation

A simple visual evaluation is possible without any need for special technical equipment. Compare the inoculated samples with the positive and negative control samples (Fig. 1).

In the presence of guaiacol-forming bacteria, the transparent to slightly yellow guaiacol is converted by an enzymatic reaction into the brown coloured complex Tetraguaiacol by the peroxidase in the detection kit.

Detection of beverage spoiling bacteria with high potential for causing off-flavours if there is a marked change of colour and the brown colouring is significantly darker compared to the negative control.

There are bacteria without and/or with very low potential for causing off-flavours if the incubation tube and negative control test have the same colour change.

Guaiacol negative samples will remain colourless.

If the change of colour is ambiguous, it is advisable to increase the incubation time of the BAT-Bouillon tubes with *Alicyclobacillus* up to 24 h in an incubator.

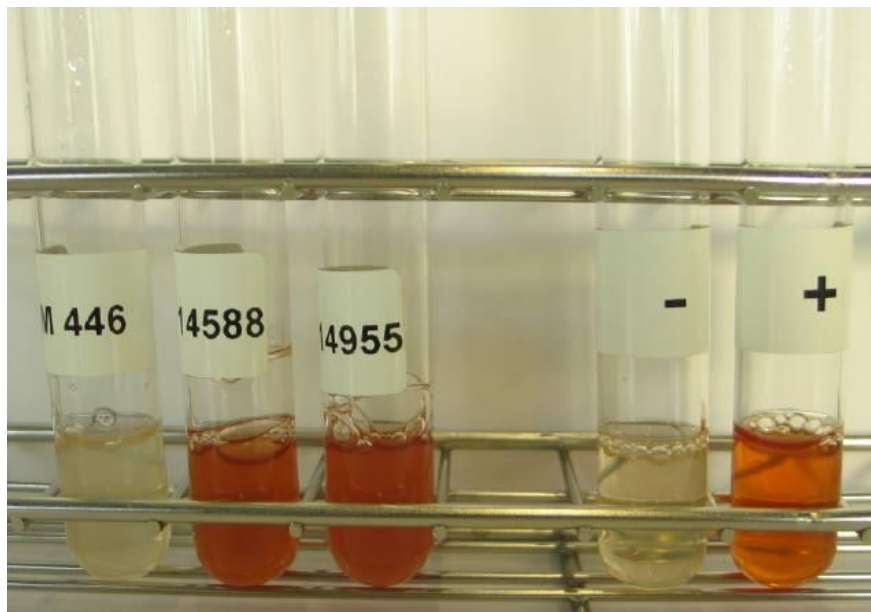


Fig. 1 Final results of the samples



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3. Storage and Packaging Information

Packaging and Content

unit	Guaiacol detection kit consisting: <ul style="list-style-type: none"> • BAT-IB: 40 test tubes (à 2 mL induction broth) • solution 1: 50 mL buffer, pH 4.0 • solution 2: 900 µL hydrogen peroxide solution (blue lid) • solution 3: Enzyme powder (brown vial and brown lid) • solution 4: 2 x Guaiacol standard (yellow lids, 1050 ppm Guaiacol)
	40 tubes (box)
unit size	approx. 30 cm x 25 cm x 20 cm / 12 in x 10 in x 8 in
unit weight	approx. 1.5 kg / 3 lbs

Storage

Store at 4-8°C/40-46°F according to product specification.
 Store under dry and dark conditions. The ready-made enzyme solution (solution 3) has to be used within 3-4 months. Do not freeze product.

Waste Disposal

No dangerous good.
 No hazardous material.
 Please consider your local waste regulations.
 Not inoculated media 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

Keep away from sources of ignition.

4. Related products

Investigation material	Relevant target microorganisms	Product	Format	Description	Packaging	pH (±0,2)	Incubation T [°C]	Item no.
Beverage containing juice pH < 4.5 Fruit juices Fruit juice concentrates Compounds Sugar	Alicyclobacillus spp.	BAT-A	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
		BAT-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
		Guaiacol Detection Kit	Detection kit	Enzymatic test kit for detection of off-flavour forming <i>Alicyclobacillus</i> spp.	Incl. 40 tubes (box)		43-47	2.04737.991