

Direct *Alicyclobacillus* (ACB) Vial

Product No. ACB-109

Instructions for use in Soleris Instrument



Soleris® vial uninoculated (left) and inoculated vial (right).

The Direct *Alicyclobacillus* Vial rapidly detects guaiacol producing *Alicyclobacillus* contamination in beverages and raw materials. The vial has an assay time of 48 hours for most applications. As organisms grow in the broth medium, the carbon dioxide (CO₂) produced diffuses through a membrane layer into a soft agar plug containing a dye indicator. The color change in the dye is read by the Soleris® instrument. The membrane layer also serves as a barrier, eliminating product interference with the reading frame.

Materials Required:

1. ACB-109, Direct *Alicyclobacillus* (ACB) Medium Vial
2. Water bath capable of 80°C ± 1°C.

Dependent on Sample Tested:

1. Sterile distilled or deionized water or *Alicyclobacillus* Medium
2. Sterile membrane filtration apparatus and 0.45 µm membrane filters

Guaiacol Production Confirmation:

1. Guaiacol Detection Kit – Cosmo Bio Co., Ltd., Catalog No. KOY-08921
2. 12 X 75 mm clear glass or similar tubes

Vial Specifications

1. Vial pH is 4.0 ± 0.2
2. Vial sample capacity up to 1.0 mL

Vial Preparation

1. Remove ACB-109 vials from 2–8°C storage and allow to equilibrate to room temperature.

Sample Preparation

1. Non-filterable and concentrated products
 - a. Prepare a 1:10 dilution of the sample by adding 10 grams or mL of sample to 90 mL of sterile distilled water, sterile deionized water or *Alicyclobacillus* Medium.
 - b. Heat shock the diluted sample for 10 minutes at 80 ± 1°C.
 - c. Cool to room temperature by immediately placing in an ice bath.
2. Filterable products
 - a. Heat shock 100 mL of the sample for 10 minutes at 80 ± 1°C.
 - b. Cool to room temperature by immediately placing in an ice bath.
3. Samples should be heat shocked in a container that can withstand the high, 80 ± 1°C temperature.
4. Samples prepared in *Alicyclobacillus* Medium may be pre-incubated at 45 ± 1°C for 24-28 hours prior to Soleris vial inoculation.

Inoculation of Vial

1. Non-filterable and concentrated products
 - a. Inoculate the vial with no more than 1.0 mL of the sample to be tested.
 - b. Cap the vial and gently invert 3 time to mix sample. Keep cap tight.
 - c. Insert the vial into the Soleris instrument set at 43°C or as indicated by the trainer. The incubation temperature and test duration can be optimized for different product types. It is not recommended to adjust the parameters without consulting Neogen Technical Services.
2. Filterable Products
 - a. Filter sample through a sterile membrane filtration apparatus using a 0.45 µm membrane filter.
 - b. Transfer member filter to ACB-109 vial.
 - c. Cap the vial and gently invert 3 time to mix sample. Keep cap tight.
 - d. Insert the vial into the Soleris instrument set at 43°C or as indicated by the trainer. The incubation temperature and test duration can be optimized for different product types. It is not recommended to adjust the parameters without consulting Neogen Technical Services.

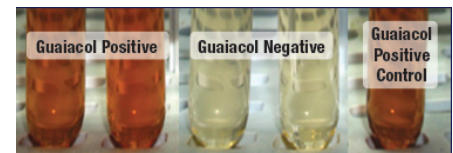
Algorithm Utilized:

Test	Threshold	Skip	Shuteye	Duration	Temperature
ACB-109	8	2	25	48 Hours	43°C

Guaiacol Production Confirmation (Optional)

Confirmation of guaiacol positive *Alicyclobacillus* can be performed from a positive Soleris Direct *Alicyclobacillus* (ACB-109) Vial

1. From a positive Soleris Direct *Alicyclobacillus* (ACB) Medium Vial, invert to mix and transfer 2.0 mL of the broth medium into an empty 12x75 mm tube.
2. Add reagents 1, 2 and 3 from the Guaiacol Detection Kit
 - a. Add 1.0 mL of Reagent 1 into each tube.
 - b. Add 0.020 mL of Reagent 2 into each tube.
 - c. Add 0.020 mL of Reagent 3 into each tube.
3. Gently mix contents of the vial.
4. Let stand for 5–10 minutes and observe for a color change.
5. Add reagents (step 2) to a separate 12x75 mm tube and 2.0 mL from an uninoculated vial for a negative control.
6. A positive reaction will turn the vial a dark brown color.



Disclaimers:

Information provided is based on validation procedures that Neogen performed in Neogen laboratories. Deviation from procedures is possible, but should be discussed with Neogen Technical Services.

Appearance of the vials should be inspected prior to use.

If shuteye detections, are observed the threshold may need to be adjusted based on the product matrix. Certain product matrices may require parameter adjustments, including increased test duration. For more information contact Neogen Technical Services.