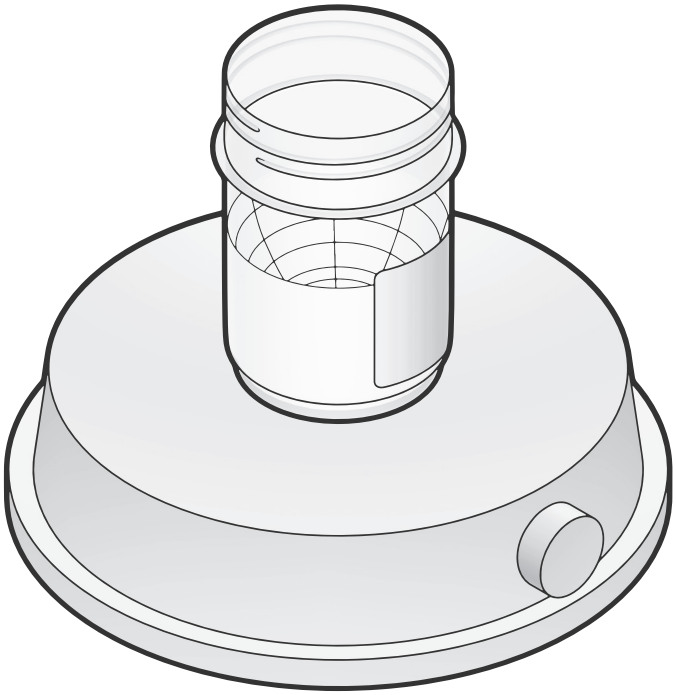

foodproof®

# StarPrep® Two Kit

## *ALICYCLOBACILLUS*

## PRODUCT INSTRUCTIONS

Documentation for the rapid extraction of
*Alicyclobacillus* DNA for direct use in PCR

Product No. KIT230177

**food**proof®

# StarPrep® Two Kit

*Alicyclobacillus*

Product No. KIT230177
42 mL volume

Store kit at 15 to 25 °C
For testing of food
and environmental samples

**PRODUCT INSTRUCTIONS**
Revision A, August 2024

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# 1. OVERVIEW

The foodproof® StarPrep® Two *Alicyclobacillus* Kit is designed for the rapid preparation of DNA from *Alicyclobacillus* for direct use in PCR. The extracted DNA can be used directly in the PCR application. The StarPrep Two Lysis Buffer eliminates the need for hazardous organic extractions or chaotropic agents. In addition, the reduced number of handling steps saves time.

## 1.1 General Information

### Number of Reactions

The kit is designed for 96 reactions.

### Storage Conditions

Store at 15 to 25 °C.
The components of the foodproof StarPrep Two *Alicyclobacillus* Kit are guaranteed to be stable through the expiration date printed on the label.

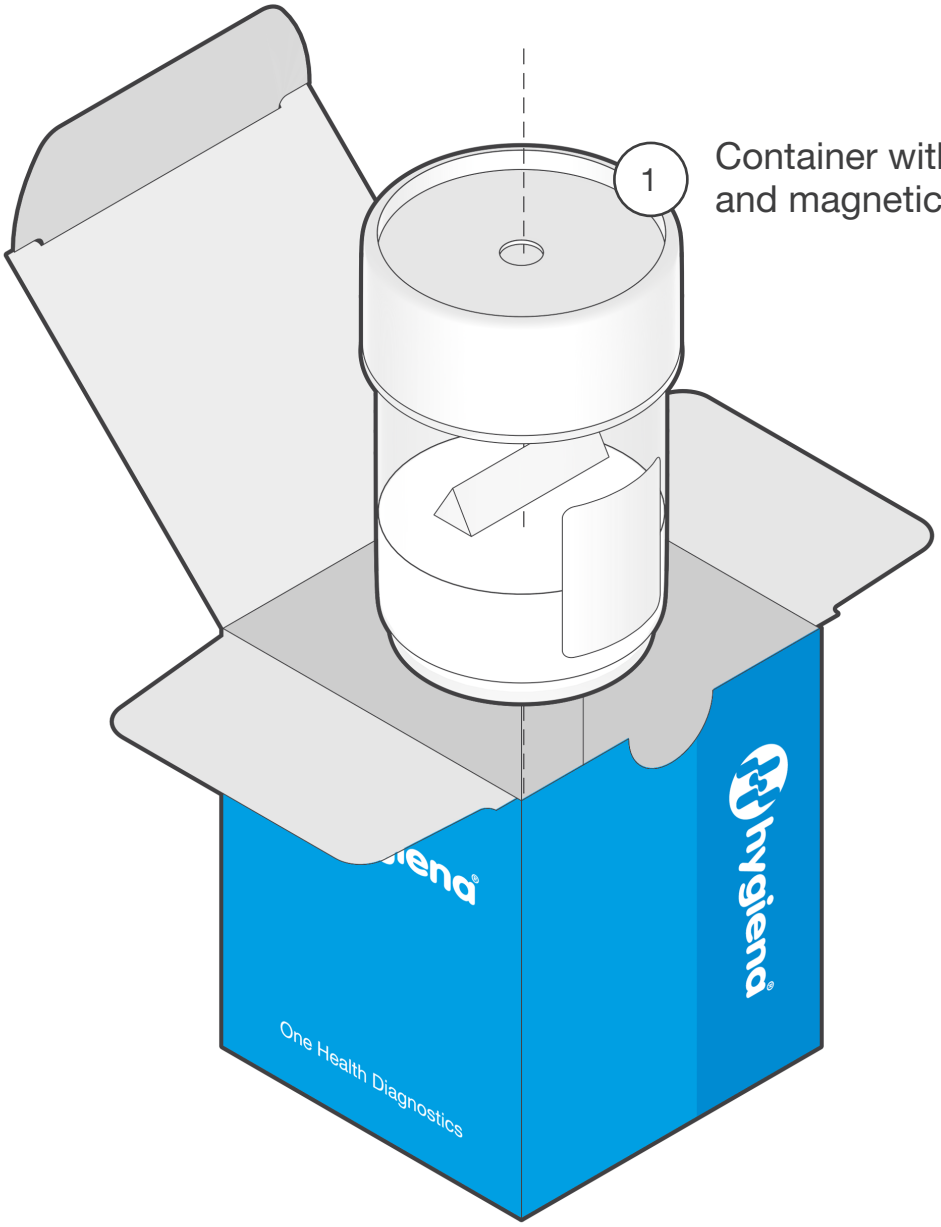
## 1.2 Applicability

The Lysis Buffer is optimized for the preparation of various types of sample material, including enrichment cultures and direct samples. The sample volume varies depending on which matrix is being tested. The quality of the DNA obtained with the Lysis Buffer is suitable for any PCR application.

## 1.3 Kit Contents

A schematic representation of the foodproof StarPrep Two *Alicyclobacillus* Kit with all its components.

### KIT230177




1 Container with 42 mL lysis buffer and magnetic stir bar

# 2. INSTRUCTIONS

This section provides all information for a seamless DNA extraction from a variety of matrices.

## 2.1 Required Material

Most of the required equipment and reagents are available through Hygiena®. Please contact us for further information.

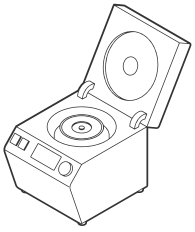
*It is highly recommended only to use the materials described below to guarantee the robustness of the method.*

### Reagents

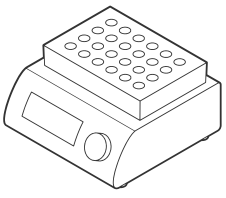
☐ **1 M Tris, pH 8**
*Not provided by Hygiena*

### Equipment

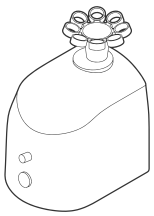
☐ Standard tabletop **microcentrifuge** capable of a 13,000 × g centrifugal force
*e.g., Micro Star 21*



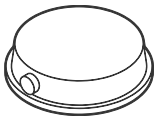
☐ **Heating unit** suitable for 1.5 mL tubes
*e.g., AccuBlock™ - Labnet with heating block*



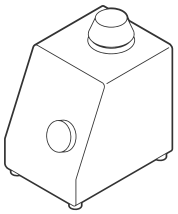
☐ Unit for **mechanical cell disruption** suitable for working with 1.5 mL reaction tubes
*e.g., Mortexer™ - Benchmark Scientific*
*or Disruptor Genie® - Scientific Industries*



☐ **Magnetic stirrer**
*e.g., Color squid IKAMAG® - IKA®-Werke*



☐ **Vortex mixer**
*e.g., Vortex-Genie® - Scientific Industries*

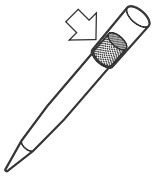


## 2.2 Precautions and Preparations

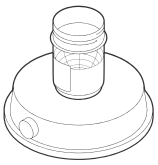
Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces and use a biosafety cabinet whenever aerosols might be generated.

For more information, please refer to the appropriate material safety data sheet (SDS). The SDS is available online at www.hygiena.com/sds.

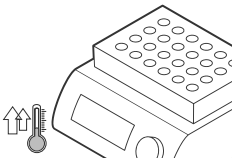
☐ Always use filter tips in order to avoid cross-contamination.



☐ Mix thoroughly when pipetting the buffer for sample preparation.
It is not recommended to use more than 96 reactions. The container must retain some of the reagent. Do not use any more reagent once the minimum level mark on the container has been reached. The mark indicates the minimal allowed pipetting level while the stirrer is not in use.



☐ Set the heating unit to 95 to 100 °C.



## 2.3 Workflows

This manual contains protocols for the extraction of *Alicyclobacillus* bacteria. Please note that specific protocols are available for other target organisms. These can be downloaded from our website at www.hygiena.com:

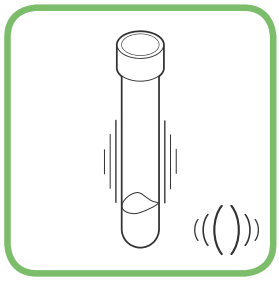
***Listeria***
***Clostridium***
***Legionella***
**Beer-spoiling bacteria**
**Spoilage yeast**
**Yeast and mold**
***Aspergillus***

The following procedure describes DNA extraction from *Alicyclobacillus* enrichment cultures and bacterial colonies.

# 2.3.1 EXTRACTION PROCEDURE: STANDARD

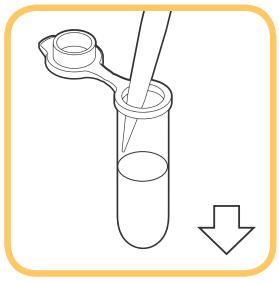
This protocol describes the DNA isolation from an enrichment culture. This fast protocol needs only a few pipetting steps.

For enrichment, the following is recommended: Dilute sample 1:10 in BAT Medium (10 mL sample in 90 mL BAT broth). Activate spores according to International Fruit and Vegetable Juice Association (IFU) method no. 12 (MM12) instructions: Heat to 80 °C for 10 min, then enrich for up to 5 days at 45 °C.



## 1. SHAKE SAMPLE

Shake enrichment culture gently and let the suspension settle for 5 to 10 min.



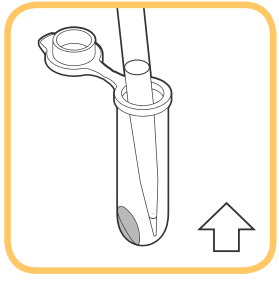
## 2. ADD SAMPLE

Transfer **2,000 µL** sample (enrichment culture supernatant) to a 2.0 mL reaction tube.

## 3. CENTRIFUGE

**2 min at 500 x g.**

*Note: If necessary, centrifugation forces should be calculated according to the manual for the centrifuge used.*



## 4. TRANSFER SUPERNATANT

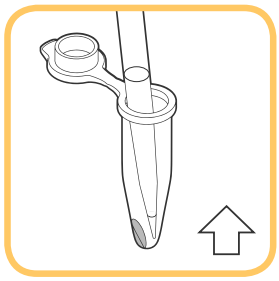
Using a pipette, transfer **1,500 µL** supernatant into a new tube.

*Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.*

## 5. CENTRIFUGE

**10 min at 8,000 x g**

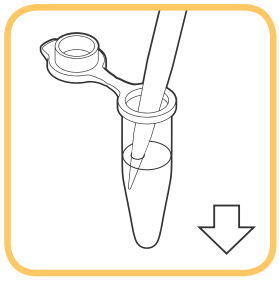
*Note: If necessary, centrifugation forces should be calculated according to the manual for the centrifuge used.*



## 6. REMOVE SUPERNATANT

Using a pipettor, carefully remove the supernatant, leaving the pellet intact.

*Note: Pipette carefully, taking care that the tip of the pipette is on the opposite side of the pellet during pipetting. Do not disturb the pellet.*



## 7. RESUSPEND PELLET

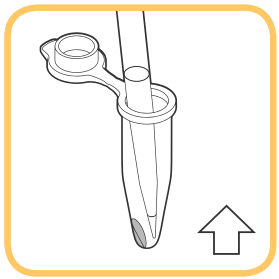
Using a pipettor, **add 1 mL 1 M TRIS Buffer (pH 8)** and resuspend pellet.

*Note: Ensure pellet is completely resuspended in the buffer.*

## 8. CENTRIFUGE

**5 min at 8,000 x g**

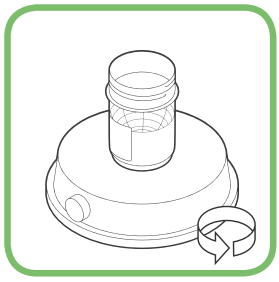
*Note: If necessary, centrifugation forces should be calculated according to the manual of the centrifuge used.*



## 9. REMOVE SUPERNATANT

Using a pipettor, carefully remove the supernatant, leaving the pellet intact.

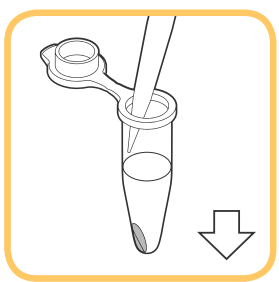
*Note: Pipette carefully, taking care that the tip of the pipette is on the opposite side of the pellet during pipetting. Do not disturb the pellet.*



## 10. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.
**Continuously mix lysis buffer at 400 rpm** on the magnetic stirrer to keep solution homogeneous.
Open the lysis buffer container.

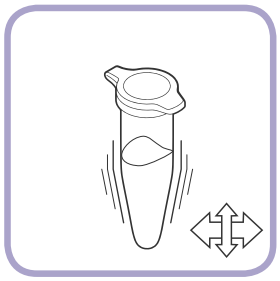
*Note: Hold the container while switching on the magnetic stirrer and during pipetting.*



## 11. ADD LYSIS BUFFER

**Transfer 300 µL lysis buffer to the sample tube** and resuspend the pellet by pipetting gently up and down 5 to 10 times.

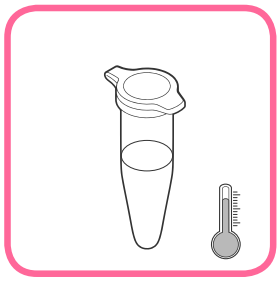
*Note: Pipette carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.*
*Use a 1,000 µL filter tip to transfer lysis buffer to the sample.*
*For optimal DNA isolation efficiency, the pellet has to be completely resuspended.*



## 12. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:
**Disruptor device: 8 min at maximum speed.**

*Note: The efficiency of disruption depends on the mechanical cell disruption unit.*



## 13. INCUBATE

**5 min at 95 to 100 °C** in a heating unit.
Carefully remove the reaction tube from the heating unit and allow the tube to COOL for **1 min at 15 to 25 °C**.

## 9. MIX

**Vortex for 2 sec.**

## 10. CENTRIFUGE

**5 min at 13,000 x g.**

*Note: If necessary, centrifugation forces should be calculated according to the manual of the centrifuge used.*

## SUPERNATANT FOR DETECTION

**Use 5 - 25 µL extract for the foodproof PCR kits (depending on sample type** (5 µL is recommonded with orange-juice/tomato concentrates or samples that may cause inhibition).
*Strictly avoid transferring fractions of the sediment to the PCR reaction because this might cause PCR inhibition.*

**For later analysis, store DNA at -15 to -25 °C.**
*After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 2 min.*

# 2.4 Troubleshooting

| Problem | Possible Cause | Recommendation |
|---|---|---|
| Extract inhibits PCR | Enrichment culture or sample contains too many PCR inhibitors. | Perform a subcultivation, e.g., 1:10 dilution in fresh enrichment broth.<br>Repeat DNA extraction with reduced sample volume. |
| | DNA extract contains too many PCR inhibitors. | Dilute DNA extract, e.g., 1:10, or reduce the amount of extracted DNA, e.g., for LyoKits 5 µL instead of 25 µL. |
| | Some of the centrifugation pellet transferred over to the PCR. | Always centrifuge the DNA sample before performing PCR.<br>Use the top of the supernatant as a PCR template.<br>Do not allow the filter tip to have contact with the pellet. |
| Low DNA yield | Improper storage of kit components. | Store kit reagents at 15 to 25°C. |
| | Enrichment culture contains substances that reduce the DNA extraction efficiency. | Perform a subcultivation or dilution, e.g., 1:10 in fresh enrichment broth. |
| | Sample contains substances that reduce the DNA extraction efficiency. | Reduce the sample volume. |
| | Not enough target organisms in enrichment culture. | Prolong the incubation phase. |
| | Pellet resuspension incomplete. | Improve resuspension by prolonged pipetting or vortexing. |
| | No or insufficient beads in the reaction. | Use correct stirring settings.<br>Do not pipette more than 96 / 192 (depending on protocol) reactions.<br>Do not use reagent below the minimal level indicated |
| | Suboptimal reaction conditions. | Ensure proper disruption and heating conditions.<br>Verify heating block at correct temperature by using a thermometer. |
| Lid of the reaction tube opens during or after heating | Reaction tube not firmly closed. | Use lid clips for closing the tubes properly.<br>Use a heating unit that enables removal of the tubes without directly touching the tube lids. |

## 2.5 Support

If you have questions or experience any problems with our products, please contact us:

**www.hygiena.com/support**

Our aim is to provide you with a solution as quickly and effectively as possible. We would also like you to contact us if you have any suggestions for improving the product or in case you would like to use our product for a different application. We highly value your feedback.

# 3. ADDITIONAL INFORMATION

## 3.1 Quality Control

All products are regularly monitored by our quality control. You can find the certificate of analysis (COA) on our website. If you would like to carry out your own quality control, you will find the analysis method described in the certificate.

## 3.2 Waste Disposal

All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For proper disposal of unused chemicals, please refer to the SDS.

## 3.3 Warranty and Disclaimer of Liability

“Limited Warranty” and “Disclaimer of Liability”: Hygiena Diagnostics GmbH warrants that this product is free from defects in materials and workmanship through the expiration date printed on the label and only if the following are complied with:

(1) The product is used according to the guidelines and instructions set forth in the product literature;

(2) Hygiena Diagnostics GmbH does not warrant its product against any and all defects when: the defect is as a result of material or workmanship not provided by Hygiena Diagnostics GmbH; defects caused by misuse or use contrary to the instructions supplied, or improper storage or handling of the product;

(3) All warranties of merchantability and fitness for a particular purpose, written, oral, expressed or implied, shall extend only for a period of one year from the date of manufacture. There are no other warranties that extend beyond those described on the face of this warranty;

(4) Hygiena Diagnostics GmbH does not undertake responsibility to any purchaser of its product for any undertaking, representation or warranty made by any dealers or distributors selling its products beyond those herein expressly expressed unless expressed in writing by an officer of Hygiena Diagnostics GmbH;

(5) Hygiena Diagnostics GmbH does not assume responsibility for incidental or consequential damages, including, but not limited to responsibility for loss of use of this product, removal or replacement labor, loss of time, inconvenience, expense for telephone calls, shipping expenses, loss or damage to property or loss of revenue, personal injuries or wrongful death;

(6) Hygiena Diagnostics GmbH reserves the right to replace or allow credit for any modules returned under this warranty.

## 3.4 Trademarks

**food**proof®, **micro**proof®, **vet**proof®, ShortPrep®, StarPrep®, RoboPrep® and LyoKit® are registered trademarks of Hygiena Diagnostics GmbH.
Hygiena® is a registered trademark of Hygiena.
Other brand or product names are trademarks of their respective holders.

## 3.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 08.1.

## 3.6 Change Index

*Revision A, August 2024:*
First version of the product instructions.
S 400 08.1 20-1 -> INS-KIT230177-9-REVA

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