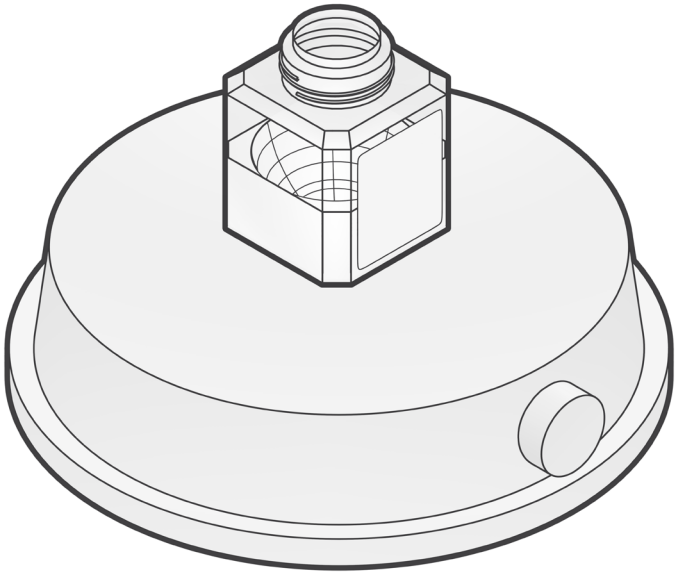


foodproof®

StarPrep® Three Kit

STEC

PRODUCT INSTRUCTIONS

Documentation for the rapid DNA extraction from
Shiga toxin–producing *Escherichia coli* for direct use in PCR

Product No. KIT230187

foodproof®

StarPrep® Three Kit

STEC

Product No.: KIT230187

21 mL volume

Store kit at 15 to 25 °C

For testing of food
and environmental samples

Approval:

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 102004

PRODUCT INSTRUCTIONS

Revision A, September 2023

TABLE OF CONTENTS

1. OVERVIEW

The foodproof® StarPrep® Three Kit is designed for the rapid preparation of DNA from Gram-negative bacteria like Shiga toxin-producing *E. coli* (STEC) for direct use in PCR. In less than 30 minutes, preparation with this lysis buffer yields PCR template DNA from enrichment cultures. The extracted DNA can be used directly in any PCR application.

The StarPrep Three Lysis Buffer eliminates the need for hazardous organic extractions or chaotropic agents. The entire DNA preparation can be performed in a single tube, minimizing handling steps and exposure to hazardous material. The reduced number of handling steps saves time and eliminating DNA-containing extract transfer steps minimizes the risk of cross-contamination.

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 Three Kit are guaranteed to be stable through the expiration date printed on the label.

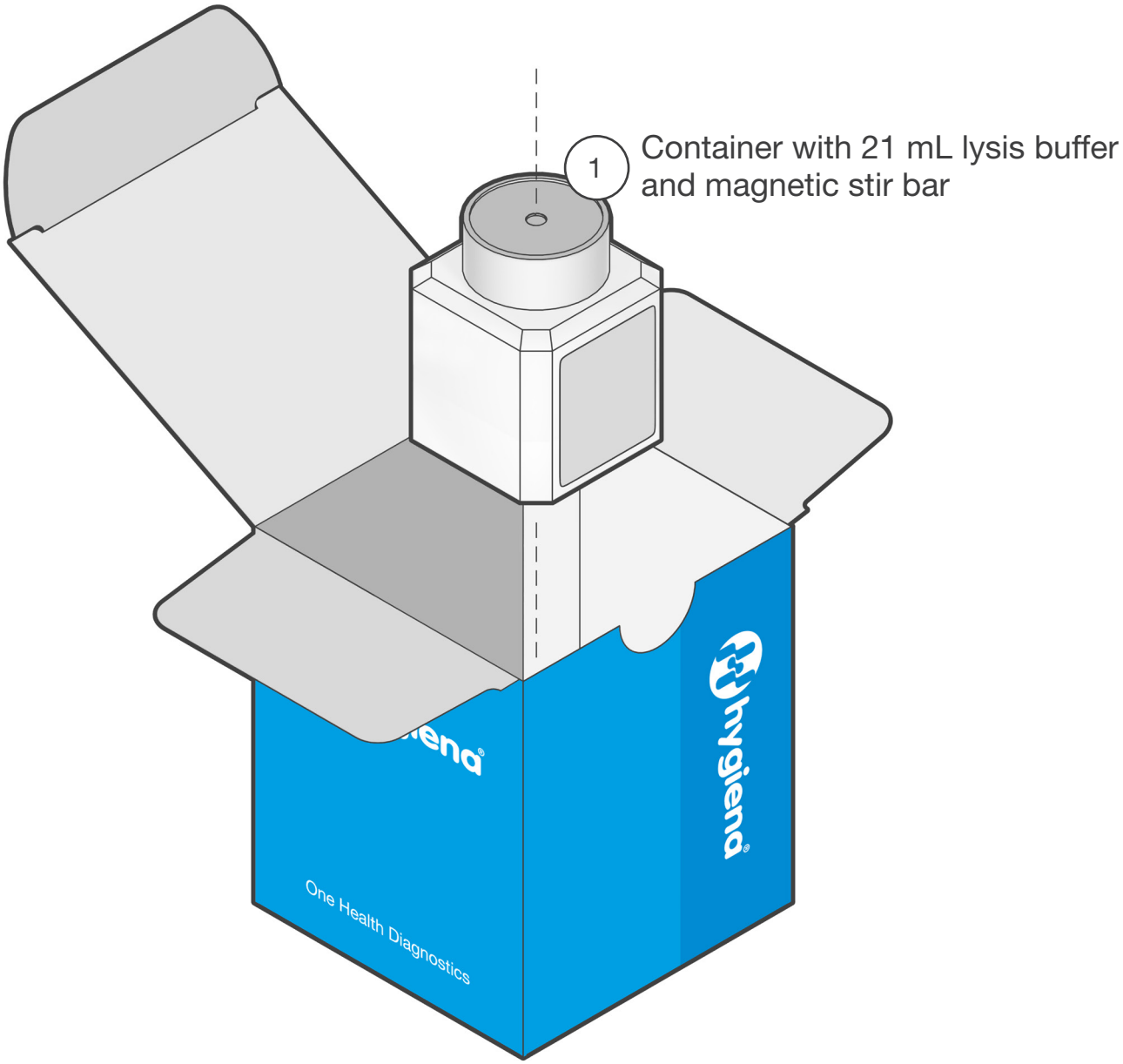
1.2 Applicability

The lysis buffer is optimized for the preparation of enrichment cultures of various types of sample material, including meat and sprouts. 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 Three Kit with all its components.

KIT230187



2. INSTRUCTIONS

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

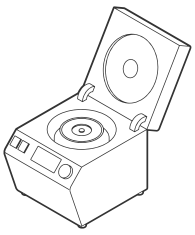
2.1 Required Material

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

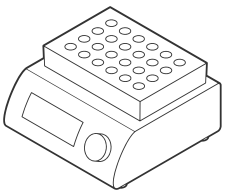
It is highly recommended to only use the materials described below to guarantee the robustness of the method.

Equipment

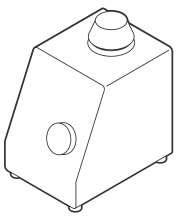
☐ Standard tabletop **microcentrifuge** capable of a 13,000 × g centrifugal force
e.g., Micro Star 21



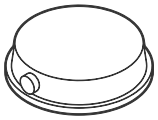
☐ **Heating unit** suitable for 1.5 mL tubes capable of a temperature range of 95 to 100 °C
e.g., AccuBlock™ - Labnet with heating block



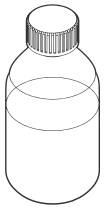
☐ **Vortex mixer**
e.g., Vortex-Genie® - Scientific Industries



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



Reagents



☐ Sterile 0.85 % **NaCl solution**
Not provided

Only for procedure B (2.3.2)

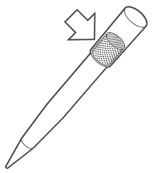
2.2 Safety and Preparations

Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats, gloves and other personal protective equipment at all times. The assay should only be used by adequately trained personnel. Properly dispose of all contaminated materials, clean and decontaminate work surfaces with an appropriate disinfectant of choice (e.g., sodium hypochlorite solution) before and after use as part of aseptic techniques. Use a biosafety cabinet whenever aerosols might be generated. In addition to cleaning workstations, work areas should be separated for the following: media preparation, sample preparation and pathogen detection. Laboratory equipment like pipettes or tubes must not circulate between workstations. When working with enrichment cultures, filter laboratory bags should be used to minimize particulates and shaking the enrichment bag or collecting large food fragments should be avoided. For fatty foods, collect the sample just below the fat layer. Never reuse kit disposables and always change serological pipettes and pipette tips between samples.

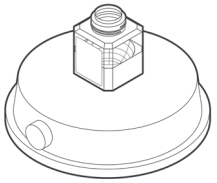
All contaminated and potentially infectious material, like enrichment cultures or food samples, should be autoclaved before disposal and eliminated according to local rules and regulations. For more information, e.g., proper disposal of unused chemicals, please refer to the appropriate safety data sheet (SDS).

The SDS is available online at www.hygiena.com/sds.

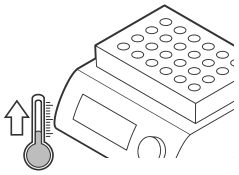
☐ Always use filter tips in order to avoid cross-contamination.



☐ Mix thoroughly while pipetting the buffer for sample preparation.



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



2.3 Workflows

The following procedures describe the DNA isolation from enrichment cultures. Compared to Extraction Procedure A, Extraction Procedure B includes an additional washing step, if the enrichment medium contains acriflavine or other fluorescent substances.

The “High Target Amount” protocol describes the DNA isolation from enrichment cultures with a high amount of target organism, and the “Colonies” protocol includes the DNA isolation from bacterial colonies.

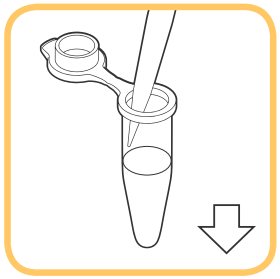
We offer an additional rapid protocol for colony confirmation in combination with the **micro**proof® Suspension Buffer (Product No. KIT 2301 78. Please refer to the product instructions).

2.3.1 EXTRACTION PROCEDURE A

This protocol describes the DNA isolation from enrichment cultures.

1. SHAKE SAMPLE

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



2. ADD SAMPLE

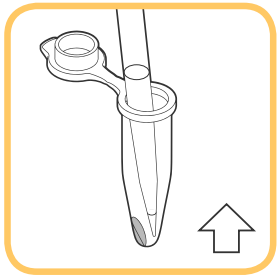
Transfer **100 µL** sample (supernatant enrichment culture, 20 to 24 h enrichment time point) to a 1.5 mL reaction tube.

Note: For 25 g meat sample enrichment cultures with 8 to 20 h enrichment time, please use 500 µL For 375 g meat sample enrichment cultures with 12 to 20 h, please use 500 µL.

3. CENTRIFUGE

5 min at 8,000 x g.

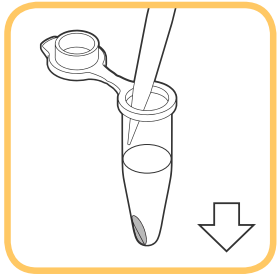
Note: If enrichment cultures are totally clear, centrifugation at ≥ 13,000 × g is recommended.



4. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

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



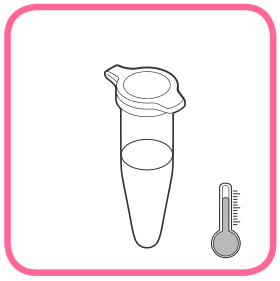
5. ADD LYSIS BUFFER

Transfer **200 µL** StarPrep Three Lysis Buffer to the sample tube.

Note: Use a magnetic stirrer (low speed) or gently shake the bottle with the lysis buffer for a short while.

6. MIX

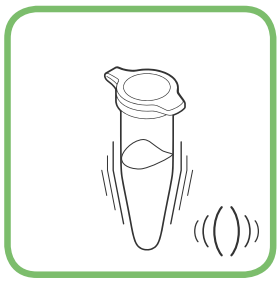
Vortex or mix by pipetting up and down until pellet has **completely resuspended**.



7. INCUBATE

10 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.

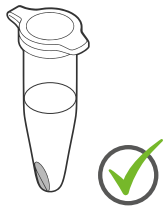


8. MIX

Vortex for **2 sec.**

9. CENTRIFUGE

2 min at 13,000 x g.



SUPERNATANT FOR DETECTION

Use 25 µL of extract for the foodproof STEC LyoKits.

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.

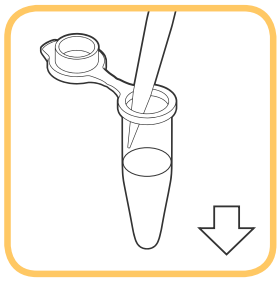
After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 2 min.

2.3.2 EXTRACTION PROCEDURE B

The following protocol describes the DNA isolation from 100 µL of enrichment culture containing acriflavine, e.g., mTSB + A (ISO/TS 13136) and mBPWp plus Acriflavin-Cefsulodin-Vancomycin (ACV) Supplement (BAM Chapter 4A).

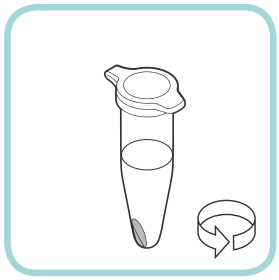
1. SHAKE SAMPLE

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



2. ADD SAMPLE

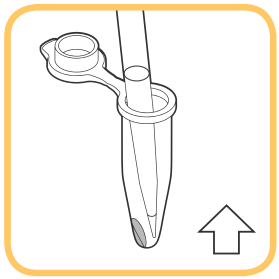
Transfer **100 µL** sample (supernatant enrichment culture) to a 1.5 mL reaction tube.



3. CENTRIFUGE

5 min at 8,000 x g.

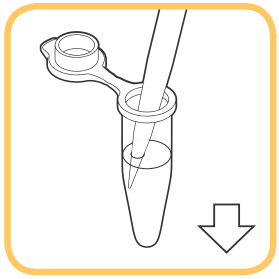
Note: If enrichment cultures are totally clear, centrifugation at ≥ 13,000 × g is recommended.



4. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

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



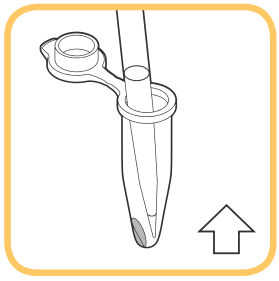
5. ADD BUFFER

Resuspend pellet in **1 mL 0.85 % NaCl**.

6. CENTRIFUGE

5 min at 8,000 x g.

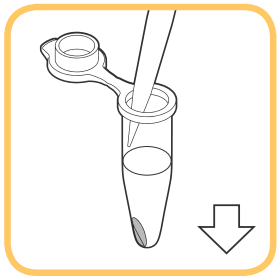
Note: If enrichment cultures are totally clear, centrifugation at ≥ 13,000 × g is recommended. Use e.g., latex beads (Sigma, Catalog-No. LB11; add 10 µL of the suspension, 1:10 in distilled water) to increase efficiency and yield a visible pellet.



7. REMOVE SUPERNATANT

Discard liquid with a pipette immediately after centrifugation and inactivate appropriately.

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



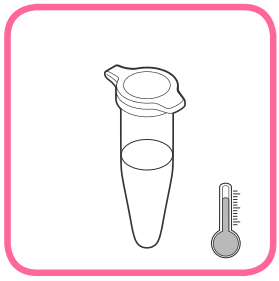
8. ADD LYSIS BUFFER

Transfer **200 µL** StarPrep Three Lysis Buffer to the sample tube.

Note: Use a magnetic stirrer (low speed) or gently shake the bottle with the lysis buffer for a short while.

9. MIX

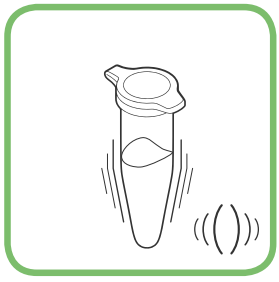
Vortex or mix by pipetting up and down until pellet has **completely resuspended**.



10. INCUBATE

10 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.

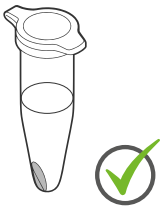


11. MIX

Vortex for **2 sec.**

12. CENTRIFUGE

2 min at 13,000 x g.



SUPERNATANT FOR DETECTION

Use 25 µL of extract for the foodproof STEC LyoKits.

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.

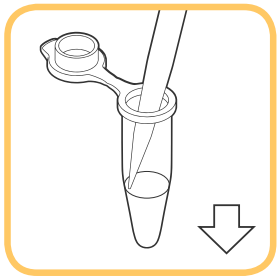
After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 2 min.

2.3.3 EXTRACTION PROCEDURE "HIGH TARGET AMOUNT"

This protocol describes the DNA isolation from enrichment cultures with a high amount of the target organisms.

1. SHAKE SAMPLE

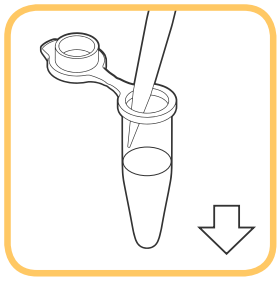
Shake enrichment culture gently and let the suspension settle for 5 to 10 min.



2. ADD LYSIS BUFFER

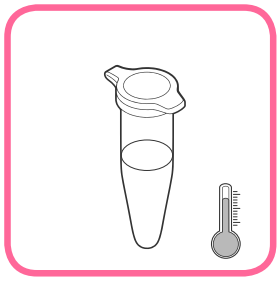
Transfer **200 µL** lysis buffer to a 1.5 mL reaction tube.

Note: Use a magnetic stirrer (low speed) or briefly shake the bottle gently before pipetting the lysis buffer to avoid sedimentation of ingredients.



3. ADD SAMPLE

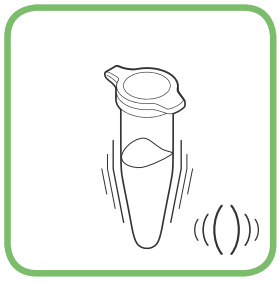
Transfer **50 µL** sample (enrichment culture supernatant) to the reaction tube containing the lysis buffer and mix briefly.



4. INCUBATE

10 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.

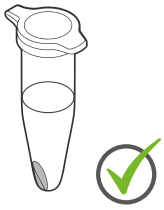


5. MIX

Vortex for 2 sec.

6. CENTRIFUGE

2 min at 13,000 x g.



SUPERNATANT FOR DETECTION

Use 25 µL of extract for the foodproof STEC LyoKits.

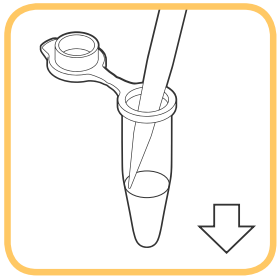
Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

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.

2.3.4 EXTRACTION PROCEDURE: “COLONIES”

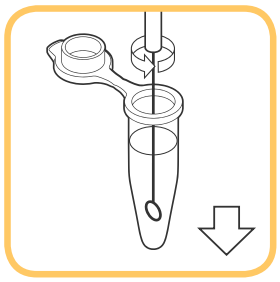
For a rapid protocol for colony confirmation, please refer to our Suspension Buffer Product Instructions (Product No. KIT230178) or contact our support.



1. ADD LYSIS BUFFER

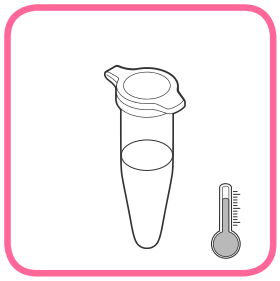
Transfer **200 µL** lysis buffer to a 1.5 mL reaction tube.

Note: Use a magnetic stirrer (low speed) or briefly shake the bottle gently before pipetting the lysis buffer to avoid sedimentation of ingredients.



2. ADD PICKED COLONIES

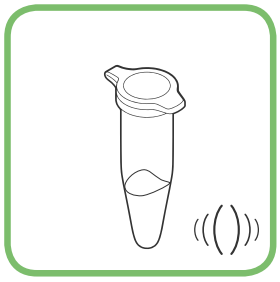
Transfer a small part of the colony with a suitable tool.
(e.g., inoculating needle) to the reaction tube containing the lysis buffer and mix by gently swirling.



3. INCUBATE

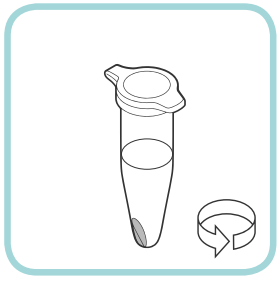
10 min at 95 to 100 °C in a heating unit.

Carefully remove the reaction tube from the heating unit and allow the tube to sit for **1 min at 15 to 25 °C**.



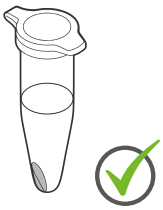
4. MIX

Vortex for 2 sec.



5. CENTRIFUGE

2 min at 13,000 x g.



SUPERNATANT FOR DETECTION

Use 25 µL of extract for the foodproof STEC LyoKits.

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. Repeat DNA extraction with a reduced sample volume. For very cloudy supernatants, a reduction of the sample volume might enhance DNA isolation efficiency.
	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 + 20 µL PCR-grade H_2O instead of 25 µl.
	Some of the centrifugation pellet transferred over to the PCR.	Always centrifuge the DNA sample before performing PCR. Use the top of the supernatant as a PCR template. Do not allow the filter tip to make contact with the pellet.
	Supernatants are not completely removed.	Remove supernatants completely.
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. Important note: this will also reduce sensitivity.
	Not enough target organisms in enrichment culture.	Prolong the incubation phase.
	Enrichment culture is totally clear	Centrifuge at ≥ 13,000 × g. Use latex beads (Sigma, Catalog-No. LB11; add 10 µL of the suspension, 1:10 in distilled water).
	Pellet resuspension incomplete.	Improve resuspension by prolonged pipetting or vortexing.
	Suboptimal reaction conditions.	Ensure proper heating conditions. Verify correct temperature of the heating block with a thermometer.
Lid of the reaction tube opens during or after heating.	Reaction tube not firmly closed.	Ensure that all reaction tubes are firmly closed before heating. Use lid clips for closing the tubes properly. 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 General Information

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.

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.

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.

Trademarks

3.2 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 18.

3.3 Change Index

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Version 2, February 2022:
Rebranding.

Revision A, September 2023
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