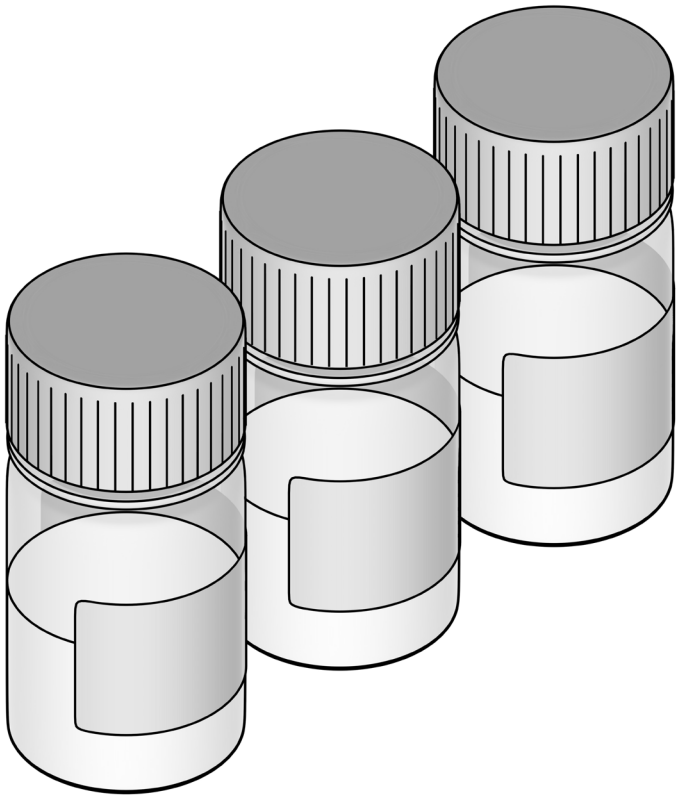


foodproof®

StarPrep® One Kit

PRODUCT INSTRUCTIONS

Documentation for the rapid extraction of DNA from gram-negative bacteria for direct use in PCR

Product No. KIT230175 / KIT230176

foodproof®

StarPrep® One Kit

Product No.

KIT230175
21 mL volume

KIT230176
105 mL volume

Store kit at 15 to 25 °C

For testing of food
and environmental samples

Approvals:

PERFORMANCE TESTED
AOAC
RESEARCH INSTITUTE
LICENSE NUMBER 120301

MICROVAL®
NEN

NordVal International

PRODUCT INSTRUCTIONS

Revision A, September 2023

TABLE OF CONTENTS

1. OVERVIEW

The foodproof® StarPrep® One Kit is designed for the rapid preparation of DNA from gram-negative bacteria like *Salmonella* or *Cronobacter* for direct use in PCR. For testing with a high background of dead bacteria, an additional live-dead treatment with Reagent D can be performed. In less than 30 minutes, preparation with this lysis buffer yields PCR template DNA from 100 µL (or more) of enrichment cultures. The extracted DNA can be used directly in any PCR application. The StarPrep One 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 100 reactions (KIT 2301 75) or 500 reactions (KIT 2301 76).

Storage Conditions

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

1.2 Applicability

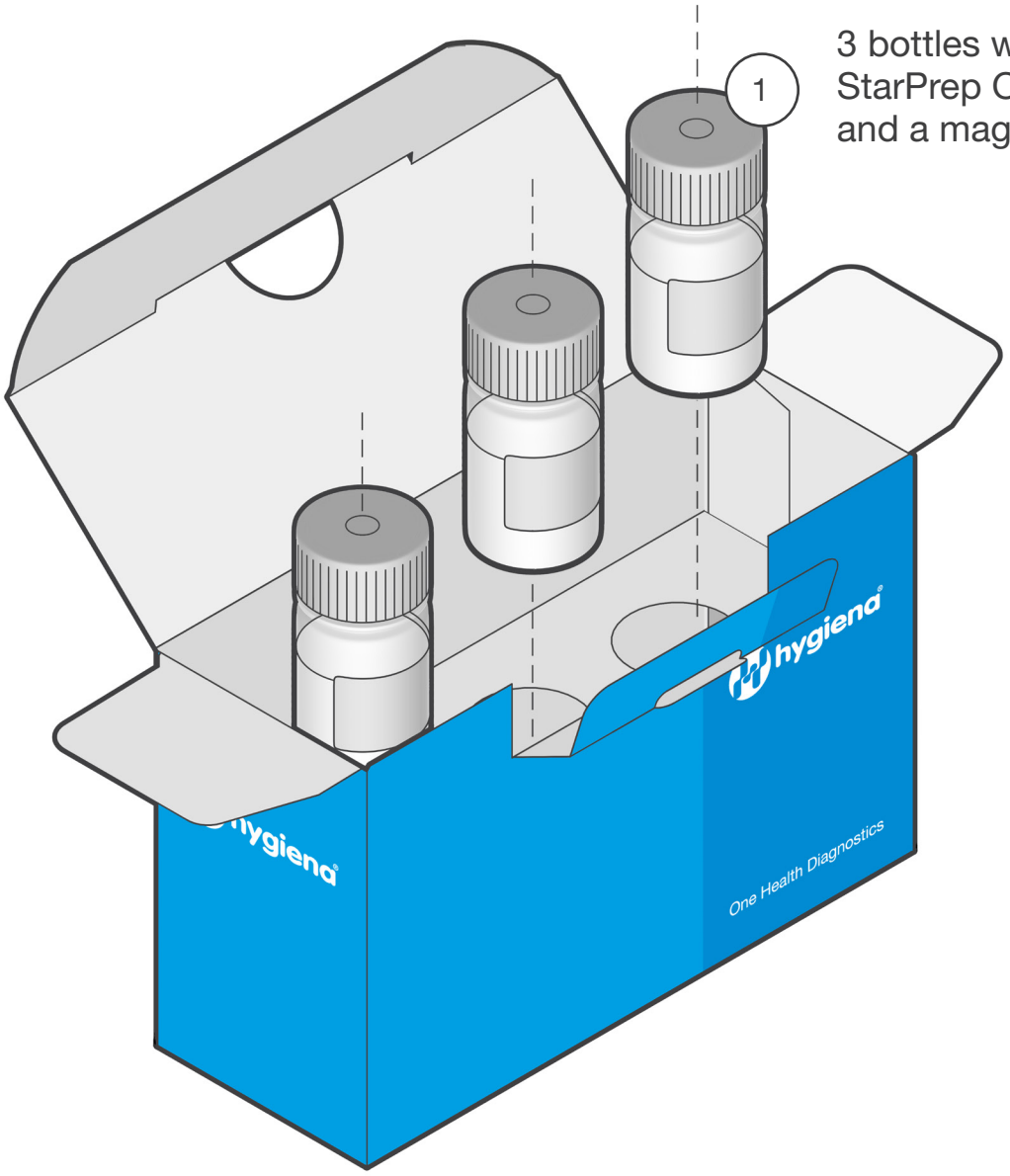
The lysis buffer can be used to prepare gram-negative bacterial DNA from 100 µL (or more) of enrichment culture.

The lysis buffer is optimized for the preparation of enrichment cultures of various types of sample material. The quality of the DNA obtained is suitable for any PCR application. The foodproof StarPrep One Kit is MicroVal-, NordVal- and AOAC RI-validated for a variety of foods.

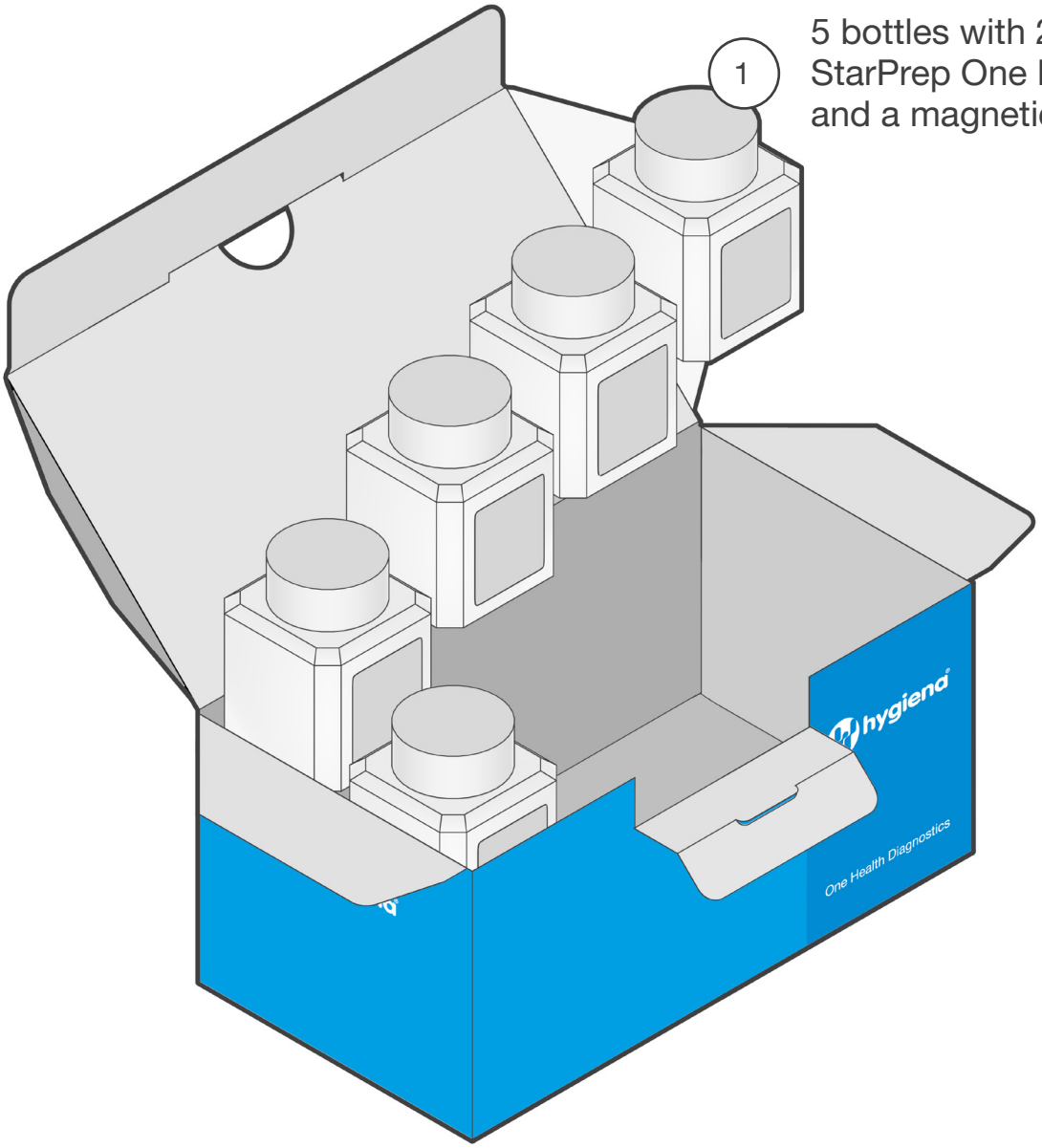
1.3 Kit Contents

A schematic representation of the foodproof StarPrep One Kit for the standard and the large version with all its components.

KIT 230175



KIT 230176



5 bottles with 21 mL StarPrep One Lysis Buffer and a magnetic stir bar

2. INSTRUCTIONS

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

2.1 Required Material

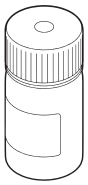
Most of the required equipment and reagents are available through Hygiena®.
Please contact us for further information.

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

Reagents

☐ **Reagent D**

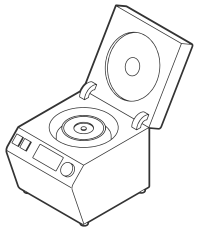
Order No. KIT230001



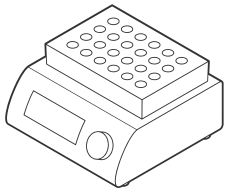
Only for extraction procedure B: Live/Dead (2.3.2)

Equipment

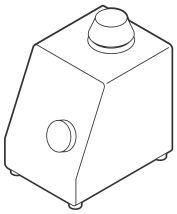
☐ Standard tabletop **microcentrifuge** capable of a 13,000 × g centrifugal force
e.g., Micro Star 21



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

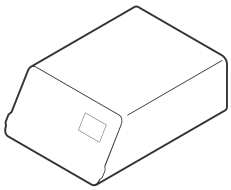


☐ **Vortex mixer**
e.g., Vortex-Genie® - Scientific Industries



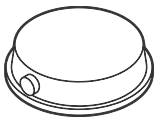
☐ **D-Light**, high-power blue LED incubation unit

Only for extraction procedure B: Live/Dead (2.3.2)



Recommended:

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

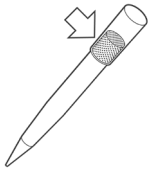


2.2 Precautions and Preparations

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.

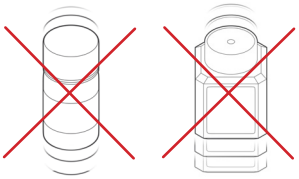
For more information, please refer to the appropriate material safety data sheet (SDS). The SDS is available online at www.hygiena.com/sds.

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

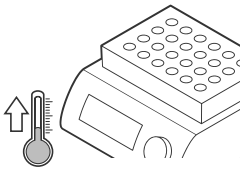


☐ To avoid foam formation of the lysis buffer, do not shake the bottles up and down.

Mix thoroughly while pipetting the buffer. For mixing, use a magnetic stirrer at low speed to move the stir bar in the bottle (high speed might cause the bottle to topple over). Alternatively, mix the bottle contents before every pipetting step by moving it in horizontal circular motions on the lab bench.

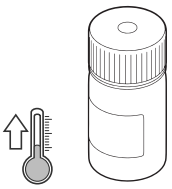


☐ Set the heating unit to 95 to 100 °C.



☐ Thaw the Reagent D prior to use.

Avoid more than three freeze-thaw cycles. If necessary, aliquots can be prepared and stored according to the Reagent D manual. Avoid extended exposure to light.



Only for extraction procedure B: Live/Dead (2.3.2)

2.3 Workflows

The following procedures describe the DNA isolation from enrichment cultures and bacterial colonies. Compared to the “Standard” protocol, the “Live/Dead” protocol includes an additional Reagent D step to eliminate dead cell DNA. Protocol “Alternative 1” describes the DNA isolation from enrichment cultures with a high amount of target organism, and the “Alternative 2” protocol includes the DNA isolation from bacterial colonies.

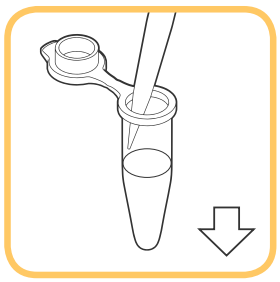
The foodproof StarPrep One Kit can be used in combination with medium and high throughput protocols. Using a multichannel pipette and 8-tube strips, multiple samples can be processed simultaneously. For protocols, please refer to the foodproof StarPrep One 8-Strip Kit (KIT230183), which also includes the necessary reagents and consumables.

2.3.1 EXTRACTION PROCEDURE A: STANDARD

This protocol describes the DNA isolation from 100 µL enrichment culture for the detection of gram-negative bacteria.

1. SHAKE SAMPLE

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



2. ADD SAMPLE

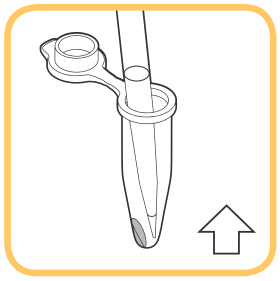
Transfer **100 µL** sample (supernatant) to a 1.5 mL reaction tube.

Note: For very cloudy supernatants, a reduction of the sample volume (e.g., 50 µL) might enhance the DNA isolation efficiency.

3. CENTRIFUGE

5 min at 8,000 x g.

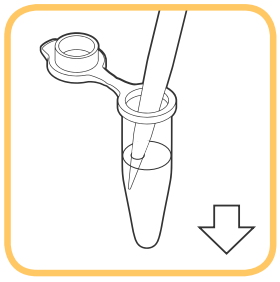
Note: If the enrichment cultures are totally clear, centrifugation at > 13,000 x g is recommended.



4. REMOVE SUPERNATANT

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

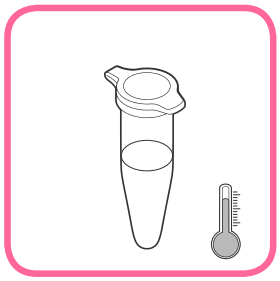
Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



5. ADD LYSIS BUFFER

Transfer **200 µL** lysis buffer to the sample tube and resuspend the pellet by vortexing or by pipetting gently up and down.

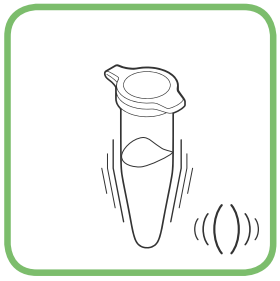
Note: For optimal DNA isolation efficiency, pellet has to be completely resuspended. Use a magnetic stirrer (low speed) or briefly shake the bottle gently before pipetting the lysis buffer to avoid sedimentation of ingredients.



6. 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**.



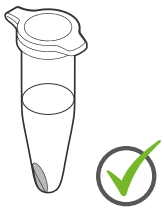
7. MIX

Vortex for 2 sec.

8. CENTRIFUGE

2 min at 13,000 x g.

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



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

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.

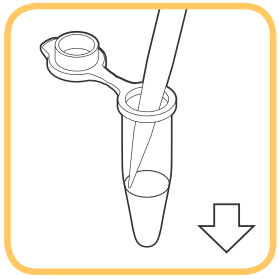
After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 2 min.

2.3.2 EXTRACTION PROCEDURE B: LIVE/DEAD

This protocol is recommended for the detection of *Enterobacteriaceae*, or *Enterobacteriaceae* in combination with other organisms, e.g., *Salmonella* or *Cronobacter*. A step for live and dead cell differentiation with Reagent D is included.

1. SHAKE SAMPLE

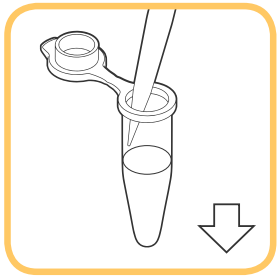
Shake enrichment culture gently and let the suspension settle for 5 to 10 min.



2. ADD REAGENT D

Transfer **300 µL** Reagent D to a transparent 1.5 mL reaction tube.

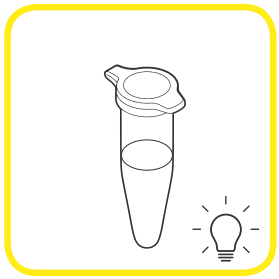
Note: Proceed immediately with the following steps of the protocol. Avoid extended exposure to light.



3. ADD SAMPLE

Transfer **100 µL** sample (enrichment culture supernatant) to the reaction tube. **Mix thoroughly** by pipetting up and down.

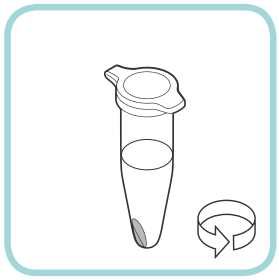
Note: For very cloudy supernatants, a reduction of the sample volume (e.g., 50 µL) might enhance the DNA isolation efficiency.



4. D-LIGHT TREATMENT

Incubate for **5 min at room temperature** in the D-Light **in the dark**.

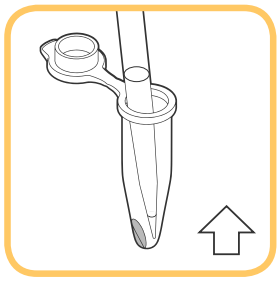
Incubate for **5 min at room temperature** in the D-Light **with light exposure.**



5. CENTRIFUGE

5 min at 8,000 x g.

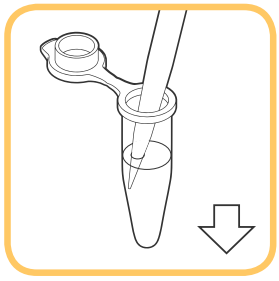
Note: If the enrichment cultures are totally clear, centrifugation at > 13,000 x g is recommended.



6. REMOVE SUPERNATANT

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

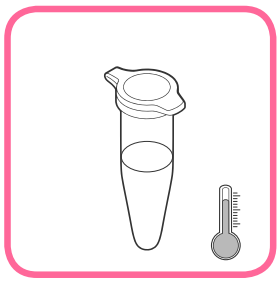
Note: Take care that the tip of the pipette is on the opposite side of the pellet during pipetting.



7. ADD LYSIS BUFFER

Transfer **200 µL** lysis buffer to the sample tube and resuspend the pellet by vortexing or by pipetting gently up and down.

Note: For optimal DNA isolation efficiency, pellet has to be completely resuspended. Use a magnetic stirrer (low speed) or briefly shake the bottle gently before pipetting the lysis buffer to avoid sedimentation of ingredients.



8. 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**.

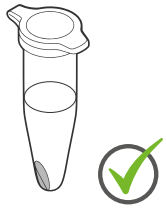
9. MIX

Vortex for 2 sec.

10. CENTRIFUGE

2 min at 13,000 x g.

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



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

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.

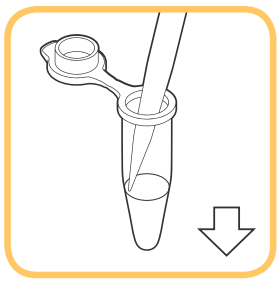
After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 2 min.

2.3.3 EXTRACTION PROCEDURE C: ALTERNATIVE 1

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

1. SHAKE SAMPLE

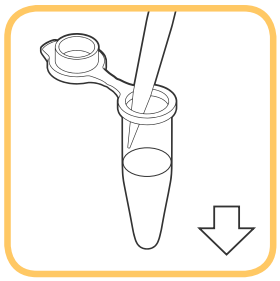
Shake enrichment culture gently and let the suspension settle for 5 to 10 min.



2. ADD LYSIS BUFFER

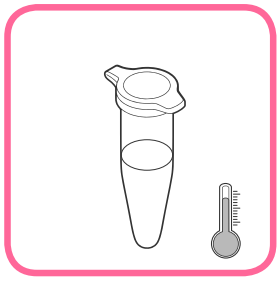
Transfer **200 µL** lysis buffer to a 1.5 mL reaction tube.

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**.

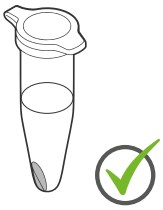
5. MIX

Vortex for 2 sec.

6. CENTRIFUGE

2 min at 13,000 x g.

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



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

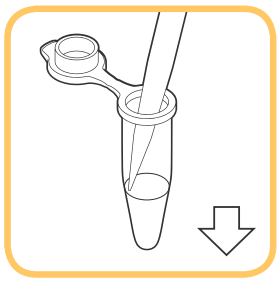
Strictly avoid transferring fractions of the sediment to the PCR reaction, because this might cause PCR inhibition.

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 D: ALTERNATIVE 2

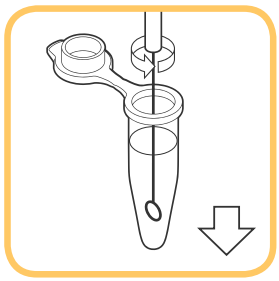
This protocol describes the DNA isolation from bacterial colonies.



1. ADD LYSIS BUFFER

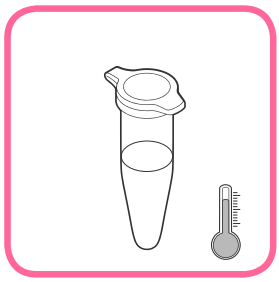
Transfer **200 µL** lysis buffer to a 1.5 mL reaction tube.

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

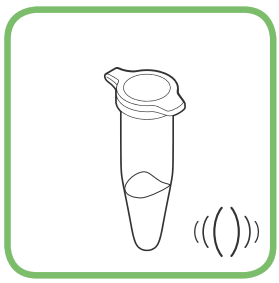
Transfer a small part of the colony with a suitable tool (e.g., inoculating needle) to the reaction tube containing the lysis buffer and mix by gently swirling.



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**.



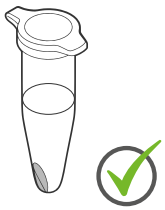
4. MIX

Vortex for 2 sec.

5. CENTRIFUGE

2 min at 13,000 x g.

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



SUPERNATANT FOR DETECTION

Use extract for the foodproof PCR kits.

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.
	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. Use the top of the supernatant as a PCR template. Do not allow the filter tip to have contact with the pellet.
	Supernatants are not completely removed.	Remove supernatants completely (e.g., after Reagent D treatment).
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.	Subculture or perform a 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.
	Suboptimal reaction conditions.	Ensure proper heating conditions. Verify heating block is at correct temperature using 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 07 (L).

3.3 Change Index

Version 1, October 2009:
First version.

Version 2, October 2010:
Adding large version of the kit.

Version 3, October 2010:
Addition of MicroVal Logo.

Version 4, July 2014:
High throughput protocol added.

Version 5, Janurary 2017:
Ultra-rapid high throughput protocol added.

Version 6, October 2019:
New document layout and content.

Version 7, February 2022:
Rebranding.

Revision A, September 2023:
New branding.
S 400 07 20 -> INS-KIT230175-76-REVA

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