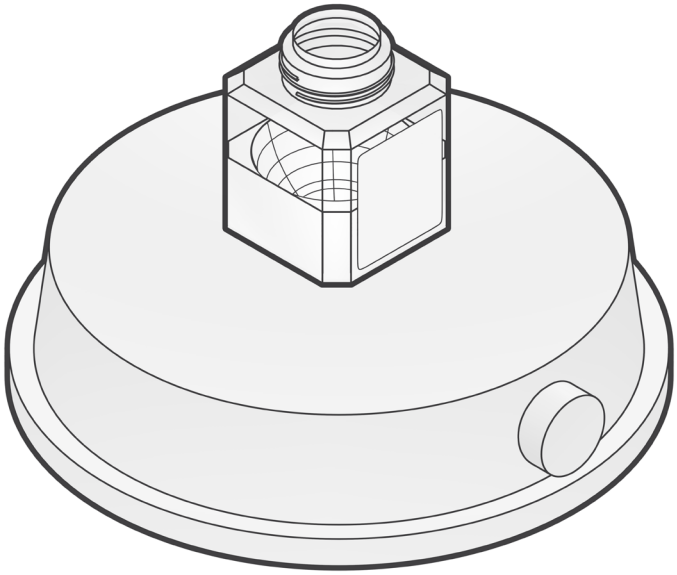


foodproof

StarPrep® Three Kit

Beverage Spoilage Bacteria

PRODUCT INSTRUCTIONS

Documentation for the rapid preparation of DNA from beverage spoilage bacteria for direct use in PCR

Product No. KIT230187

foodproof

StarPrep® Three Kit:
Beverage Spoilage Bacteria

Store kit at 15 to 25 °C
For food testing purposes
FOR *IN VITRO* USE ONLY

Product No. KIT230187
21 mL volume

Product Instructions:
Revision A, January 2024

1. OVERVIEW

The foodproof StarPrep Three Kit is designed for the rapid preparation of DNA from beverage spoilage bacteria for direct use in PCR. In less than 30 minutes, preparation with this lysis buffer yields PCR template DNA from 50 µL(or more) of enrichment culture. 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. Transfer steps of DNA containing extracts are not necessary, thus cross-contamination risks are minimized.

1.1. General Information

Number of Reactions

The kit is designed for up to 400 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.

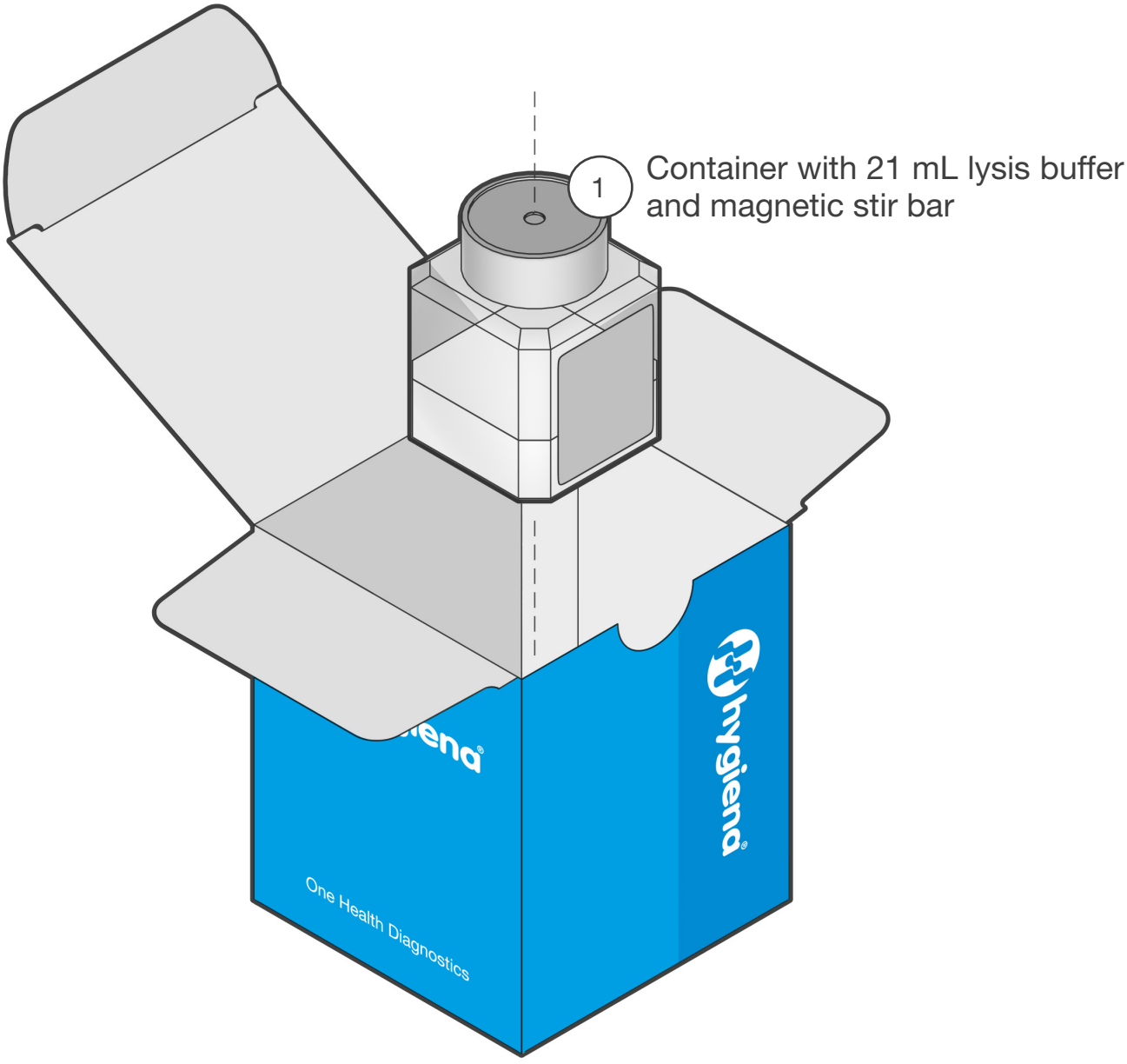
1.2. Applicability

The lysis buffer is optimized for the preparation of various types of sample material, including enrichment cultures, direct samples and filtered samples. For very cloudy supernatants, or samples containing inhibitors, a reduction of the sample volume may enhance the DNA isolation efficiency. The quality of the DNA obtained with the lysis buffer is suitable for any PCR application. Compared to StarPrep Two, the protocols have fewer handling steps, but with slightly lower sensitivity.

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.

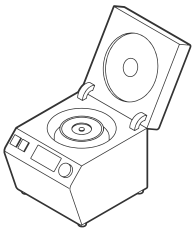
2.1. Required Material

Most of the required equipment and reagents are available through Hygiena Diagnostics. Please contact us for further information: www.hygiena.com/support

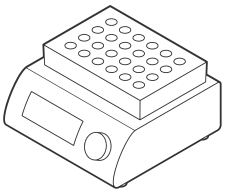
It is highly recommended only to use the materials described below to guarantee the robustness of the method.

Equipment

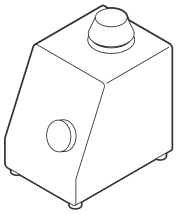
☐ Standard tabletop **microcentrifuge** capable of a 15,000 × g centrifugal force
e.g., Micro Star 17 - VWR



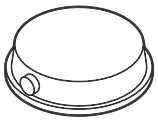
☐ **Heating unit** suitable for 1.5 mL tubes
e.g., AccuBlock™ - Labnet with heating block



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



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

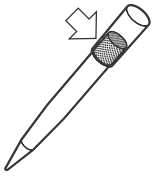


2.2. Precautions and Preparations

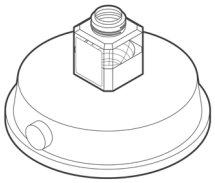
Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

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

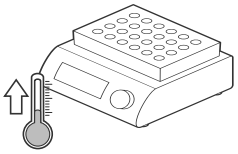
☐ Always use filter tips in order to avoid cross-contamination.



☐ Mix thoroughly while pipetting the buffer for sample preparation.
It is not recommended to use more than 400 reactions. The container must retain some of the reagent.



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



2.3. Workflows

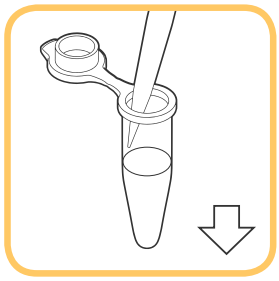
The following procedures describe the DNA isolation from enrichment cultures. Compared to the fast standard protocol, the alternative protocol includes an additional centrifugation step to reduce the amount of eukaryotic cells (e.g., yeast) in the sample.

2.3.1. EXTRACTION PROCEDURE

This protocol describes the DNA isolation from 1 mL enrichment culture.

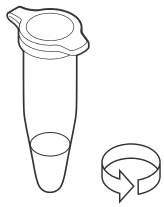
1. SHAKE SAMPLE

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



2. ADD SAMPLE

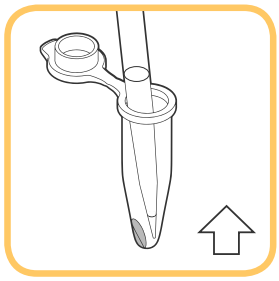
Transfer **1,000 µL** sample (supernatant enrichment culture) to a 1.5 mL reaction tube.



3. CENTRIFUGE

5 min at 15,000 x g.

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



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

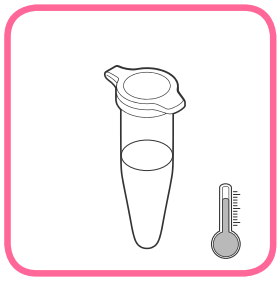
LyoKits (KIT230071/72 /73 and KIT230074/75 /76) for detection:
Transfer 150 µL StarPrep Three Lysis Buffer to the sample tube.

OR liquid kit (KIT230066) for detection:
Transfer 50 µL StarPrep Three Lysis Buffer to the sample tube.

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

6. MIX

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



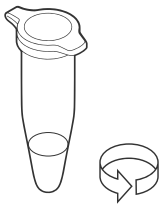
7. INCUBATE

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**.

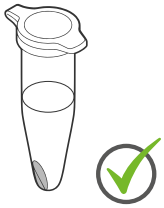
8. MIX

Vortex for 10 sec.



9. CENTRIFUGE

1 min at 15,000 x g.



SUPERNATANT FOR DETECTION

Use extract for the foodproof **PCR (Lyo)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.

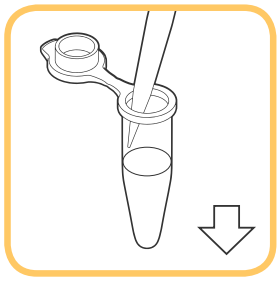
After thawing, mix briefly by vortexing and centrifuge at 15,000 × g for 1 min.

2.3.2. EXTRACTION PROCEDURE: ALTERNATIVE

This protocol describes the DNA isolation from samples with a high content of eukaryotic cells (e.g., yeast).

1. SHAKE SAMPLE

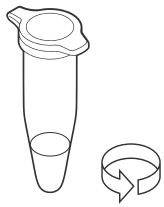
Shake enrichment culture gently and let the suspension settle for 5 to 10 min.



2. ADD SAMPLE

Transfer 1 mL sample (supernatant) to a 1.5 mL reaction tube.

Note: If the sample only has a very low fluid content it should be diluted with one volume of enrichment broth.



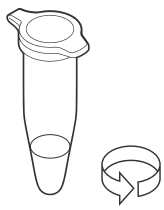
3. CENTRIFUGE

5 min at 100 x g.

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

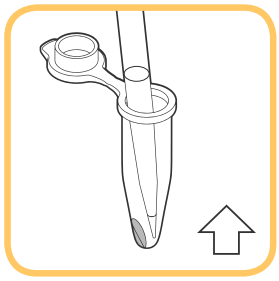
4. ADD SUPERNATANT TO A NEW TUBE

Transfer sample (supernatant) to a new 1.5 mL reaction tube.



5. CENTRIFUGE

5 min at 15,000 x g.



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

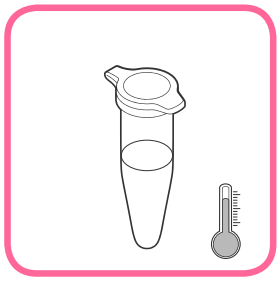
LyoKits (KIT230071/72 /73 and KIT230074/75 /76) for detection:
Transfer 150 µL StarPrep Three Lysis Buffer to the sample tube.

OR liquid kit (KIT230066) for detection:
Transfer 50 µL StarPrep Three Lysis Buffer to the sample tube.

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

8. MIX

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



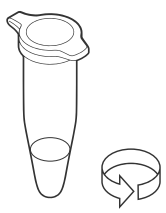
9. INCUBATE

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**.

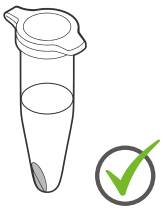
10. MIX

Vortex for 10 sec.



11. CENTRIFUGE

1 min at 15,000 x g.



SUPERNATANT FOR DETECTION

Use extract for the foodproof **PCR (Lyo)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 15,000 × g for 1 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 (e.g. 200 µLinstead of 1 mL).
	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 µLinstead of 25 µL.
	Some of the centrifugation pellet transferred over to the PCR.	Always centrifuge the DNA sample before performing PCR. Use the top portion 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.
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.	Do not pipette more than 400 reactions. Do not use reagent below the minimal level indicated.
	Suboptimal reaction conditions.	Ensure proper disruption and 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.

2.6 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.

3.2 Trademarks

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Other brand or product names are trademarks of their respective holders.

3.3 Reference Number

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

3.4 Change Index

Version 1, October 2020:
New document layout and content.

Revision A, January 2024:
Updated layout and rebranding.
S 400 18 20 3 -> INS-KIT230187-REVA

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