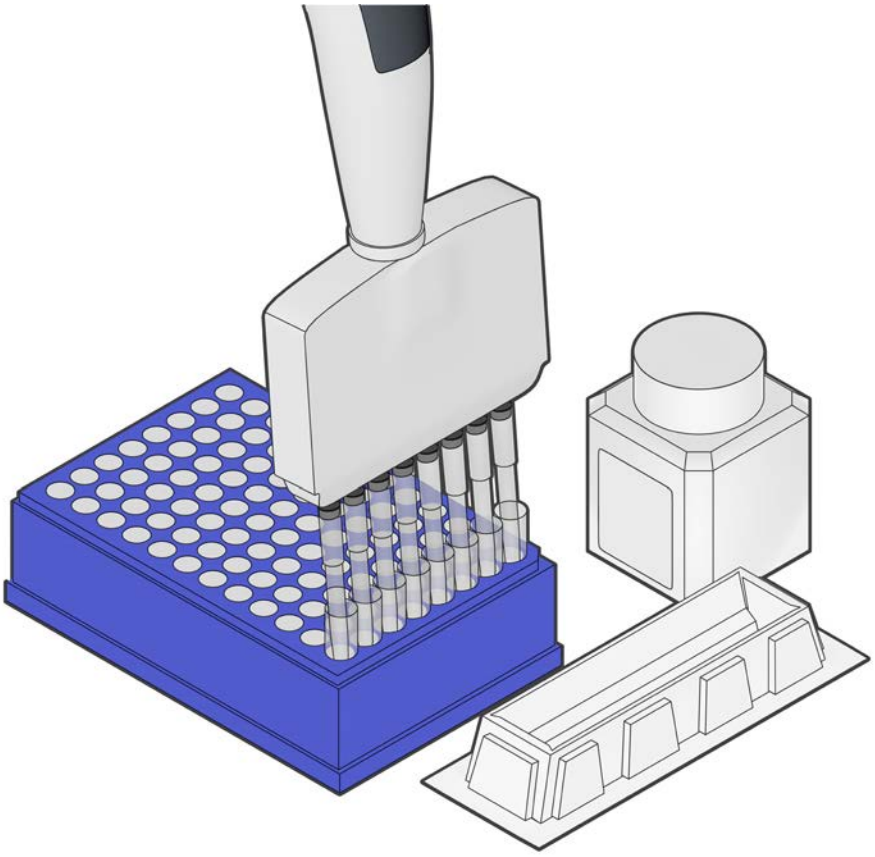

foodproof®

StarPrep® Three 8-Strip Kit

Salmonella plus *Cronobacter*

PRODUCT INSTRUCTIONS

Documentation for the rapid DNA extraction from *Salmonella* and *Cronobacter* enrichment cultures for direct use in PCR

Product No. KIT230188

foodproof®

StarPrep® Three 8-Strip Kit:
Salmonella plus *Cronobacter*

Store kit at 15 to 25 °C
FOR *IN VITRO* USE ONLY

Product No. KIT230188
Kit for 480 reactions

Product Instructions:
Revision A, September 2023

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1. OVERVIEW

The foodproof® StarPrep® Three Kit is designed for the rapid preparation of DNA from gram-negative bacteria like *Salmonella* and *Cronobacter* 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. 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 480 reactions.

Storage Conditions

Store at 15 to 25 °C.
The components of the foodproof StarPrep Three 8-Strip Kit are guaranteed to be stable through the expiration date printed on the label.

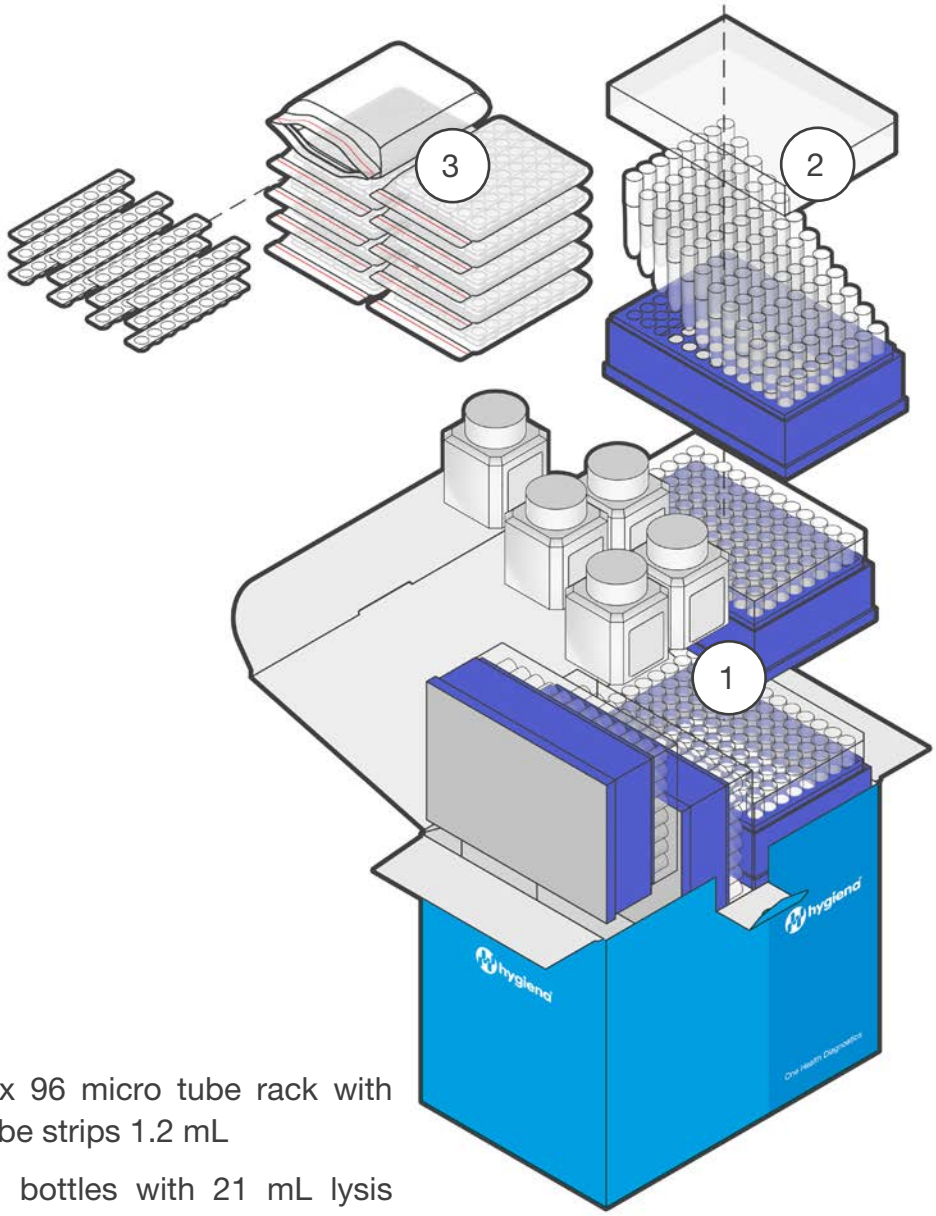
1.2 Applicability

The lysis buffer is optimized for the preparation of enrichment cultures of various types of sample material, including infant formula with and without probiotics, ingredients and production environment samples. The sample volume varies depending on which matrix is being tested. 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.

1.3 Kit Contents

A schematic representation of the foodproof StarPrep Three 8-Strip Kit with all its components.

KIT230188



1. 5x 96 micro tube rack with 8-tube strips 1.2 mL

2. 5 bottles with 21 mL lysis buffer and a magnetic stir bar

3. 10 bags with 12x 8-cap strips

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.

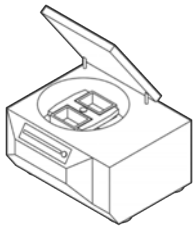
It is highly recommended only to use the materials described below to guarantee the robustness of the method.

Equipment

☐ **Multichannel pipette** and filter tips **for 50 to 1,250 µL**
e.g., 8-Channel Pipette Viaflo - INTEGRA Biosciences,
50 to 1,250 µL GripTips for Viaflo
or EP Xplorer Plus Electronic Multichannel Pipette,
50 to 1,250 µL filter tips

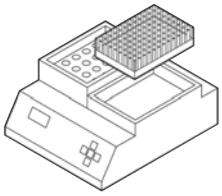
☐ **Centrifuge** with swing-out rotor for microtiter plates capable of:
> 5,400 × g centrifugal force
e.g., Sigma 4-16S including rotor

or 2,000 × g centrifugal force
e.g., Sigma 2-7 including rotor



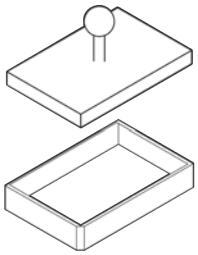
Equipment

☐ **TH 21 heating block thermostat**

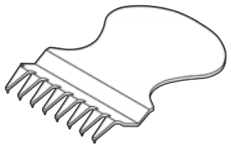


☐ Exchange block for deepwell plates for TH 21

☐ **Lid weight** with **incubation frame** for TH 21 heating block thermostat

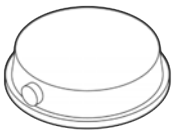


☐ **Decapper 8-strip**

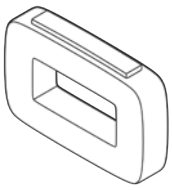


Recommended:

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

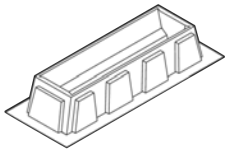


☐ **Cap installing tool**



Consumables

☐ Sterile **reservoir,** 25 mL or 100 mL



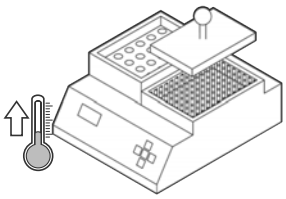
2.2 Precautions and Preparations

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 reach the required temperature of 95 - 100 °C in the tubes for the lysis step of the bacteria, the temperature of the corresponding **heating unit TH 21 has to be set to 100 °C.**



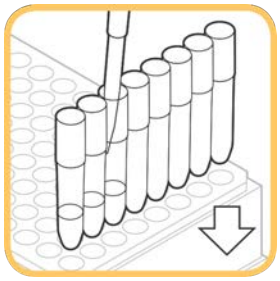
2.3 Workflow

2.3.1 EXTRACTION PROCEDURE A: STANDARD

The following protocol describes the DNA isolation from 100 µL enrichment culture.

1. SHAKE SAMPLE

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

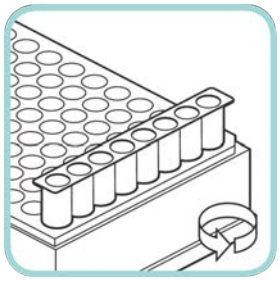


2. ADD SAMPLE

Transfer **100 µL** sample (enrichment culture supernatant) to the 8-tube strips.

3. SEAL TUBES

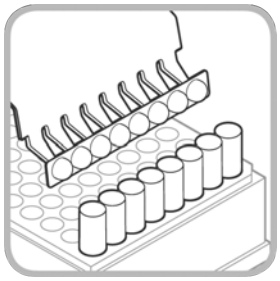
Seal the tubes with sterile cap strips.



4. CENTRIFUGE RACK

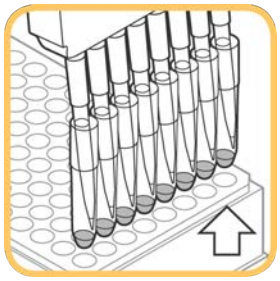
10 min at 5,400 x g (or 25 min at 2,000 x g).
Make sure the rack is not sealed with rack lid during centrifugation.

Note: Time and g-force depend on the centrifuge (please see 2.1 Required Material for more information). Set the centrifuge acceleration to maximum speed and the brake to medium. If necessary, centrifugation forces should be calculated according to the centrifuge manual used.



5. REMOVE CAPS

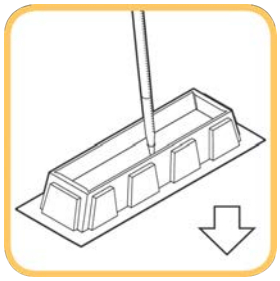
Remove and discard the 8-cap strips from the 8-tube strips.
To minimize the contamination risk, use the decapper 8-strip tool.



6. REMOVE SUPERNATANT

Remove supernatant with a multichannel pipette immediately after centrifugation, discard and inactivate appropriately.

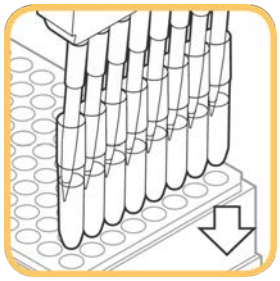
Note: Take care that the tips of the pipette in the reaction tubes are not touching the pellets.



7. PREPARE LYSIS BUFFER

Transfer required lysis buffer to a sterile reservoir. Use **200 µL** lysis buffer per sample plus 1 mL lysis buffer as dead volume.

Note: Use a magnetic stirrer (low speed) or shake the bottle with lysis buffer gently in a short time interval to avoid sedimentation of ingredients.



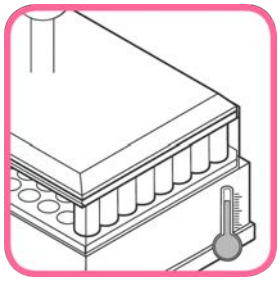
8. ADD LYSIS BUFFER

Pipet lysis buffer up and down 5 to 10 times in reservoir before using it to avoid sedimentation of ingredients.
Transfer **200 µL** lysis buffer with a multichannel pipette to each tube.
Resuspend pellets by pipetting up and down 5 to 10 times.

Note: For optimal DNA isolation efficiency, pellet has to be completely resuspended.

9. SEAL TUBES

Seal the tubes **tightly** with new sterile cap strips.



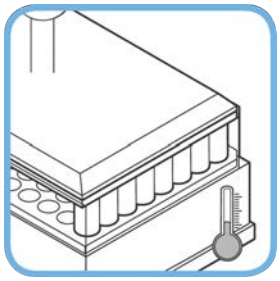
10. INCUBATE

Remove tube rack bottom and install incubation frame.

Incubate rack with tube stripes **10 - 15 min at 100 °C**
in TH 21 Heating Block for 8-tube strips.

Weight caps down with the lid weight.

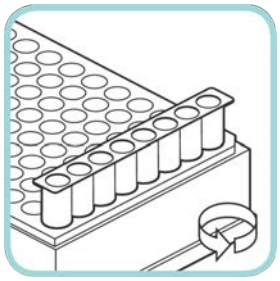
Note: To avoid removing and reinstalling the bottom, it is possible to place tube strips in an empty micro tube rack (with rack bottom removed).



11. CHILL

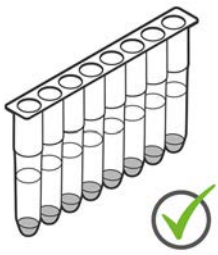
Carefully **remove** the rack with the **tube strips together with the lid weight** from the heating unit and let it **cool 3 - 5 min at room temperature**.

To avoid opening of caps, do not remove the lid weight
until the strips have cooled down.



12. CENTRIFUGE RACK

Reinstall tube rack bottom.
Centrifuge **5 min at 5,400 x g** (or 10 min at 2,000 x g).
Make sure the rack is not sealed with rack lid during centrifugation.



SUPERNATANT FOR DETECTION

Use up to 25 µL of the extract for the foodproof PCR LyoKit.

Note: 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 2,000 × g for 5 min.

Note: The sample is not purified. Proteins, RNA, and other materials remain in the sample. Long-term storage or archiving of prepared DNA samples is not recommended.

2.4 Troubleshooting

Problem	Possible Cause	Recommendation
Extract inhibits PCR	DNA extract contains too many PCR inhibitors.	For LyoKits: Use a reduced amount of extracted DNA, e.g., 20 µL PCR-grade H_2O + 5 µL DNA extract instead of 25 µL DNA extract.
	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.
	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.
	Supernatant has not been completely removed during the DNA extraction procedure.	Remove supernatant 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. For very cloudy supernatants, a reduction of the sample volume might enhance DNA isolation efficiency.
	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.
	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

The reference number and original Hygiena Diagnostics GmbH article numbers: S 400 18 L (KIT230188)

3.3 Change Index

Revision A, September 2023:
Transfer of the quick guide into more detailed and more specific product instructions.

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