



foodproof® StarPrep One 8-Strip Kit High-Throughput

PRODUCT INSTRUCTIONS

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

Product No. KIT230183

foodproof® StarPrep One 8-Strip Kit High-Throughput

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

Product No. KIT230183 Kit for 480 reactions

Product Instructions:

Revision A, April 2024



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1. OVERVIEW

The foodproof[®] StarPrep[®] One 8-Strip Kit is designed for the rapid extraction of DNA from bacteria like *Salmonella* or *Cronobacter* for direct use in PCR. Up to 96 samples can be processed in parallel. The kit generates PCR template DNA from up to 100 μ L (or more) of enrichment culture. For testing with a high background of dead bacteria, an additional live-dead treatment with Reagent D can be performed.

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. 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 One 8-Strip 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 DNA from up to a 100 μ L (or more) sample. The lysis buffer is optimized for the preparation of various types of sample material. The quality of the DNA obtained with the lysis buffer is suitable for any PCR application.

KIT230183 - StarPrep One 8-Strip Kit **OVERVIEW**



1.3 Kit Contents

A schematic representation of the foodproof StarPrep One 8-Strip Kit with all components:





2. INSTRUCTIONS

This section provides all information for a straightfoward DNA extraction from a variety of different food matrices.

2.1 Required Material

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



It is highly recommended to only use the materials described below to ensure the performance of the method.

Reagents



Only for Procedure D (2.3.4)



Equipment

Multichannel pipette and filter tips	
e.g., 8-Channel Pipette, VIAFLO - INTEGRA Biosciences; GripTips: 50 to 1,250 μL	
Or EP Xplorer Plus Electronic Multichannel Pipette; Filter Tips: 50 to 1,250 μL	and a second sec
Centrifuge with swing-out rotor for microtiter plates capable of $a > 5,400 \times g$ centrifugal force	
e.g., Sigma 4-16S including rotor Or centrifuge with swing-out rotor for microtiter plates capable of a 2,000 × g centrifugal force e.g., Sigma 2-7 including rotor	
TH 21 heating block thermostat	
Exchange block for deepwell plates for TH 21	8
Lid weight with incubation frame for TH 21 heating block thermostat	
Decapper 8-strip	
D-Light Product No. MCH230039	
Only for Procedure D (2.3.4)	\checkmark



Recommended:

Magnetic stirrer
e.g., Color squid wave - IKA[®]-Werke

] Cap installing tool





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.

Always use aersol barrier tips 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 sample preparation. For mixing, use a magnetic stirrer at	
low speed to move the stir bar in the bottle or rotate the bottle)
before every pipetting step by moving it horizontally on the lab)
bench.	

To reach the required temperature of 95 to 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.

Thaw the Reagent D prior to use.

Avoid more than three (3) freeze-thaw cycles. If necessary, aliquots can be prepared and stored according to the Reagent D manual. Avoid extended exposure to light.









Only for Procedure D (2.3.4)



2.3 Workflows

The following procedures describe the DNA isolation from enrichment culture using 8-strip tubes and multichannel pipettes.

Procedure A (PCR Lyo) and B (PCR Liquid) are more sensitive protocols, where two different extraction methods are provided depending on which type of PCR kit is used for downstream processing (for lyophilized reagents: see PCR Lyo, for liquid reagents: see PCR Liquid).

Procedure C (Rapid) is the fastest protocol suitable for less difficult matrices like milk powder.

Procedure D (Live/Dead) contains a step for live and dead cell differentiation.



2.3.1 EXTRACTION PROCEDURE A: PCR LYO

This protocol is intended for extracts that will be used in combination with foodproof LyoKits.





1. SHAKE SAMPLE

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

2. ADD SAMPLE

Transfer at least $100 \ \mu L$ sample (enrichment culture supernatant) to the 8-tube strips.

3. SEAL TUBES

Seal the tubes with sterile cap strips.



4. CENTRIFUGE RACK

Centrifuge **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 being 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.

KIT230183 - StarPrep One 8-Strip Kit 2.3.1 EXTRACTION PROCEDURE A: PCR LYO







6. REMOVE SUPERNATANT

Remove supernatant with a multichannel pipette immediately after centrifugation, discard and inactivate appropriately. Take care that the tips of the pipette in the reaction tubes are not touching the pellets.

7. PREPARE LYSIS BUFFER

Dilute the needed lysis buffer **2:1 with sterile ddH**₂**O** in a sterile reservoir: 200 μ L lysis buffer + 100 μ L H₂O per sample plus an additional 1 mL lysis buffer as dead volume.

Note: Use a magnetic stirrer (low speed) or mix the bottle with lysis buffer gently immediately before use to avoid sedimentation of ingredients.

8. ADD LYSIS BUFFER

Pipette lysis buffer up and down 5 to 10 times in reservoir before using it to avoid sedimentation of ingredients.

Transfer **300 µL** diluted 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 microtube 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.



KIT230183 - StarPrep One 8-Strip Kit 2.3.1 EXTRACTION PROCEDURE A: PCR LYO





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 μ L of the extract for the respective foodproof PCR kit.

Note: Strictly avoid transferring fractions of the sediment to the PCR reaction as 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.



2.3.2 EXTRACTION PROCEDURE B: PCR LIQUID

This protocol is intended for extracts that will be used in combination with foodproof kits (liquid).





1. SHAKE SAMPLE

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

2. ADD SAMPLE

Transfer at least $100 \ \mu L$ 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 being 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.

KIT230183 - StarPrep One 8-Strip Kit 2.3.2 EXTRACTION PROCEDURE B: PCR LIQUID





6. REMOVE SUPERNATANT

Remove supernatant with a multichannel pipette immediately after centrifugation, discard and inactivate appropriately. 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 mix the bottle with lysis buffer gently immediately before use to avoid sedimentation of ingredients.

8. ADD LYSIS BUFFER

Pipette 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 strip. Resuspend pellets by pipetting up and down 5 to 10 times.

Note: For optimal DNA isolation efficiency, pellet must 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 of the bottom, tube strips can be placed in an empty microtube 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.



KIT230183 - StarPrep One 8-Strip Kit 2.3.2 EXTRACTION PROCEDURE B: PCR LIQUID





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 5 μ L of the extract for the respective foodproof PCR kit.

Note: Strictly avoid transferring fractions of the sediment to the PCR reaction as 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.



2.3.3 EXTRACTION PROCEDURE C: RAPID

This protocol is intended for rapid high-throughput extraction in combination with foodproof kits.

It is not suitable for all types of enrichment cultures. Please contact Hygiena Diagnostics GmbH for further information.



1. SHAKE SAMPLE

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



2. PREPARE LYSIS BUFFER

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

Note: Use a magnetic stirrer (low speed) or gently mix the bottle with lysis buffer immediately before use to avoid sedimentation of ingredients.



3. ADD LYSIS BUFFER

Pipette 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.



4. ADD SAMPLE

Transfer **25 to 50 \mu L** sample (enrichment culture supernatant) to the 8-tube strips.



5. SEAL TUBES

Seal the tubes tightly with sterile cap strips.

KIT230183 - StarPrep One 8-Strip Kit 2.3.3 EXTRACTION PROCEDURE C: RAPID







6. 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, tube strips can be placed in an empty microtube rack (with rack bottom removed).

7. 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.

8. CENTRIFUGE RACK

Reinstall tube rack bottom.

Centrifuge **5 min at 2,000 x g**.

The rack must not be sealed with rack lid for 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 being used.

SUPERNATANT FOR DETECTION

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





2.3.4 EXTRACTION PROCEDURE D: LIVE/DEAD

This protocol is recommended for the detection of *Enterobacteriaceae*, or *Enterobacteriaceae* in combination with other parameter, e.g., *Salmonella* or *Cronobacter*. A step for live and dead cell differentiation with Reagent D (Product No. KIT230001) is included.





C C

1. SHAKE SAMPLE

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

2. ADD SAMPLE

Transfer $100\ \mu\text{L}$ sample (enrichment culture supernatant) to the 96 deep well plate.

3. PREPARE REAGENT D

Transfer an adequate volume of **Reagent D** into a sterile reservoir: Use **300** μ L per sample and an additional **1 mL** as dead volume.

Note: The lights in the clean bench must be switched off. Proceed immediately with the following steps of the protocol. Avoid extended exposure to light.

4. ADD REAGENT D AND MIX

Transfer **300 µL Reagent D** with a multichannel pipette to each well of the deep well plate. **Resuspend pellets** by pipetting up and down 5 times.

Note: For optimal DNA isolation efficiency, pellet must be completely resuspended. For uptake of Reagent D and mix, pipette with maximum speed of the automatic pipette. Proceed immediately with the following steps of the protocol. Avoid extended exposure to light.



5. D-LIGHT TREATMENT

Place the 96 deep well plate in the **D-Light** unit. Incubate first **in the dark for 5 min** and subsequently **expose to light for 5 min** at room temperature in the D-Light unit.

KIT230183 - StarPrep One 8-Strip Kit 2.3.4 EXTRACTION PROCEDURE D: LIVE/DEAD





6. TRANSFER VOLUME

First resuspend 5 times and then transfer the whole volume (400 µL) with a multichannel pipette from the 96 deep well plate to 8-tube strips.



7. SEAL TUBES

Seal the 8-tube strips tightly with sterile cap strips.



8. CENTRIFUGE

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



9. 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.



10. REMOVE SUPERNATANT

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

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



11. 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 gently mix the bottle with lysis buffer immediately before use to avoid sedimentation of ingredients.







12. ADD LYSIS BUFFER AND MIX

Pipette 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 must be completely resuspended.





13. SEAL TUBES

Seal the tubes tightly with new sterile cap strips.

14. 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, tube strips can be placed in an empty microtube rack (with rack bottom removed).

15. 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.



16. CENTRIFUGE

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 respective foodproof PCR kit. 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 $2,000 \times g$ for 10 min. Note: The sample is not purified. Proteins, RNA, and other materials remain in the sample. Long-term storage or archival of prepared DNA samples is not recommended.



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.
		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.	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.
	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 or not enough weight exerted on the caps of the tube strips.	Ensure that all reaction tubes are firmly closed before heating.
		Weigh the caps down during heating and do not remove the weight until the tubes have cooled down.



2.5 Support

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



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.



3.1 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

foodproof[®], microproof[®], vetproof[®], 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.

3.3 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 14 L

3.4 Change Index

Version 1, April 2018: First version.

Version 2, January 2019: Information for additional equipment added.

Version 3, November 2019: New document design.

Version 4, April 2021: Protocol for "Live/Dead" added.

Revision A, April 2024 Additional Rebranding updates, product number changes and formating. S 400 14L 20 -> INS-KIT230183-REVA

Hygiena®

Camarillo, CA 93012 USA diagnostics.support@hygiena.com

Manufactured by

Hygiena Diagnostics GmbH

Hermannswerder 17 14473 Potsdam Germany

www.hygiena.com