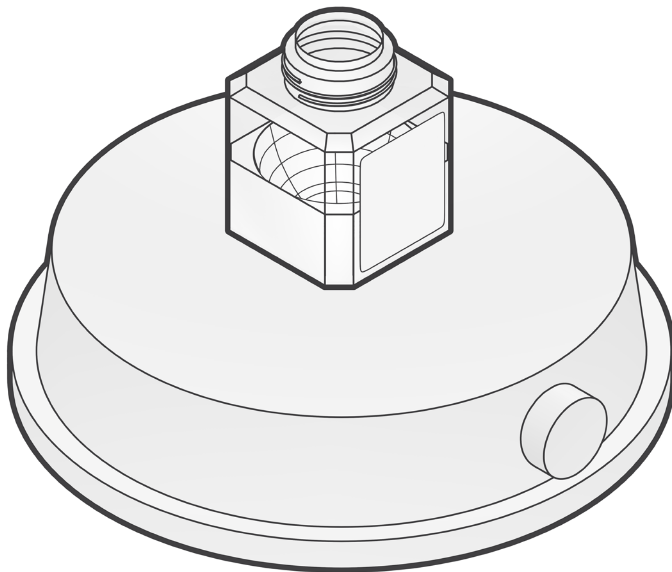
hygiena®



foodproof®

StarPrep® Three Kit

Vibrio

PRODUCT INSTRUCTIONS

Documentation for the rapid DNA extraction from *Vibrio* enrichment cultures for direct use in PCR

Product No. KIT230187

foodproof®

StarPrep® Three Kit

Vibrio

Product No.: KIT230187
21 mL volume

Store kit at 15 to 25 °C
For testing of food
and environmental samples

Approval:

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 *Vibrio* 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.

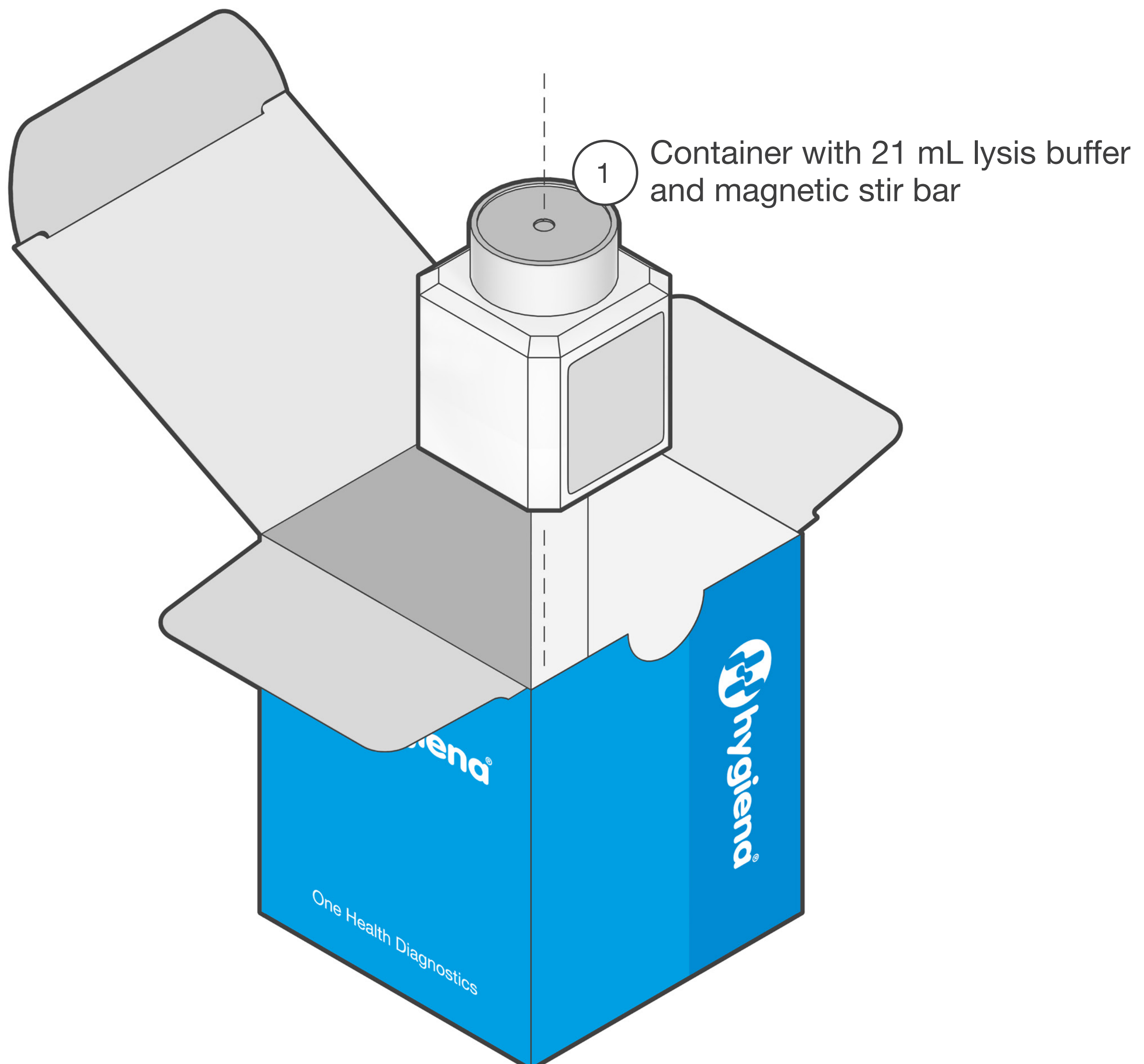
1.2 Applicability

The lysis buffer is optimized for the preparation of all relevant raw and processed seafood matrices such as whole squid, raw oysters or salmon as well as other matrices. The rapid and easy sample preparation includes a live/dead discrimination step using Reagent D, eliminating false-positive results from dead *Vibrio*. The quality of the DNA obtained with the lysis buffer is suitable for any PCR application. The foodproof StarPrep Three Kit is AOAC-RI validated in combination with the foodproof *Vibrio* Detection LyoKit for fish and seafood.

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.

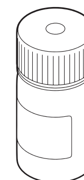
2.1 Required Material

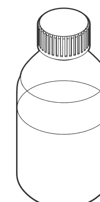
Most of the required equipment and reagents are available through Hygiena®.
Please contact us for further information.

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

Reagents

☐ **Reagent D**
Order No. KIT230001 (30 mL)
Order No. KIT230003 (15 mL)

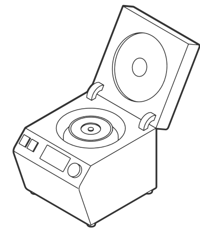




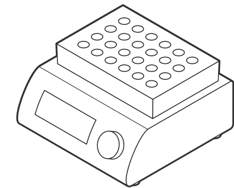
☐ Sterile **alkaline saline peptone water** (ASPW / APW)
Not provided by Hygiena

Equipment for procedure A: Standard

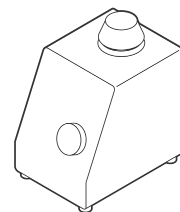
☐ Standard tabletop **microcentrifuge** capable of a 13,000 × g centrifugal force
e.g., Micro Star 21



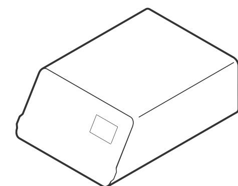
☐ **Heating unit** suitable for 1.5 mL tubes
e.g., AccuBlock™ - Labnet with heating block



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

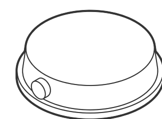


☐ **D-Light**, high-power blue LED incubation unit



Recommended:

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



Equipment for procedure B: High Throughput

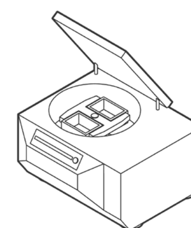
☐ **Multichannel pipette** and filter tips **for 50 to 1,250 µL**
e.g., 8-Channel Pipette Viaflo - INTEGRA Biosciences, 50 to 1,250 µL with GripTips for Viaflo

or EP Xplorer Plus Electronic Multichannel Pipette, 50 to 1,250 µL filter tips

☐ **Multichannel pipette** and filter tips **for 5 to 100 µl**
e.g., EP Xplorer Plus Electronic Multichannel Pipette, 5 to 100 µL filter tips

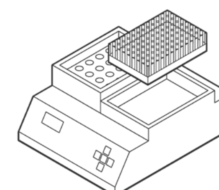
☐ **Centrifuge** with swing-out rotor for microtiter plates capable of a 2,000 × g centrifugal force

e.g., Sigma 2-7 including rotor

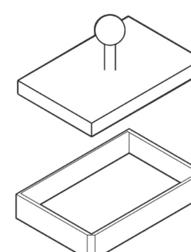


☐ **TH 21 heating block thermostat**

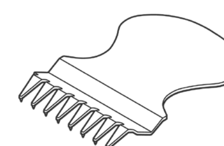
☐ **Exchange block** for deepwell plates for TH 21



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

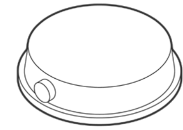


☐ **Decapper 8-strip**

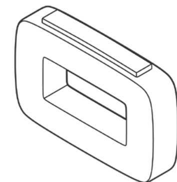


Recommended:

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

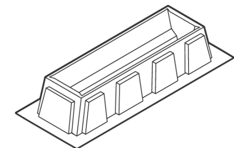


- [ ] **Cap installing tool**

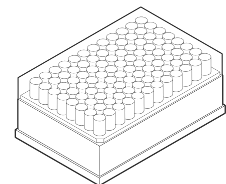


Consumables for procedure B: High Throughput

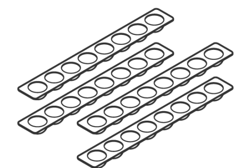
- [ ] **Sterile reservoir**, 100 mL



- [ ] **8-strip tubes, 1.2 mL** - 12 x (96 well)



- [ ] **8-Cap strips for micro tube rack**

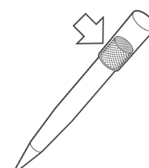


2.2 Precautions and Preparations

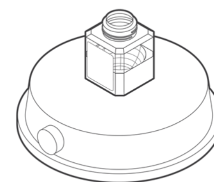
Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces and use a biosafety cabinet whenever aerosols might be generated.

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

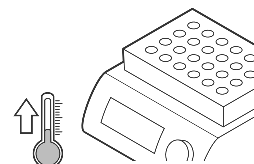
☐ Always use filter tips in order to avoid cross-contamination.



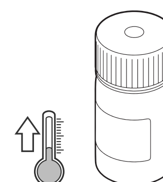
☐ Mix thoroughly while pipetting the buffer for sample preparation.
It is not recommended to use more than 96 reactions.
The container must retain some of the reagent.



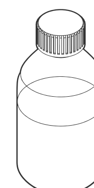
☐ Set the heating unit to 95 to 100 °C.



☐ 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.



☐ Prepare an appropriate batch of ASPW / APW



2.3 Workflows

The following procedures describe the DNA isolation from enrichment cultures. Depending on sample size, two protocols for small sample quantities (Procedure A: Standard) and high sample quantities (Procedure B: High Throughput) are available. Both protocols include a live/dead discrimination step using Reagent D. The high-throughput protocol uses 8-strip tubes and multichannel pipettes and is recommended when more than 16 samples are processed.

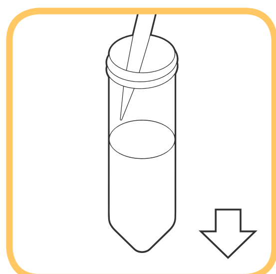
We offer an additional rapid protocol for colony confirmation in combination with the microproof® Suspension Buffer (Product No. KIT 2301 78. Please refer to the product instructions for details).

2.3.1 EXTRACTION PROCEDURE A: STANDARD

The following protocol describes the DNA isolation from 50 µL of enrichment culture. It is recommended when performing ≤ 16 isolations. Depending on sample matrices, Reagent D treatment (step 2 to 9) may be omitted.

1. SHAKE SAMPLE

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



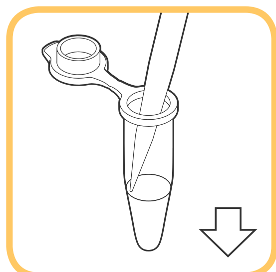
2. PREPARE PREMIX

Mix 350 µL ASPW / APW and 100 µL Reagent D per sample for a total of n samples in a sterile reservoir. Add an additional volume to adjust for pipetting errors:

(n + 1) x 350 µL ASPW / APW + (n + 1) x 100 µL Reagent D.

For example, for five DNA extractions, mix 2.1 mL ASPW / APW (6 x 350) with 600 µL Reagent D (6 x 100).

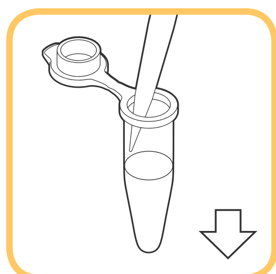
Note: Avoid extended exposure to light.



3. ADD PREMIX TO NEW TUBES

Disperse an aliquot of **450 µL** of the premix to each 1.5 mL tube.

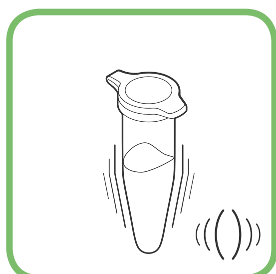
Note: Avoid extended exposure to light.



4. ADD SAMPLE

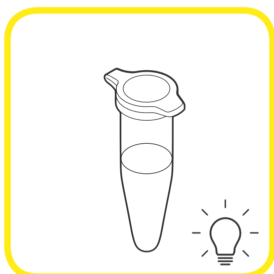
Transfer **50 µL** sample (enrichment culture supernatant) to a prefilled reaction tube.

Note: Transfer of all samples should be achieved within 5 minutes.
Avoid extended exposure to light.



5. MIX

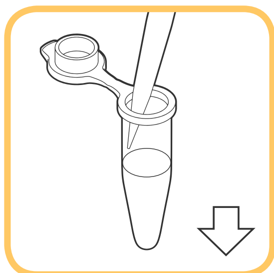
Vortex for **2 sec.**



6. D-LIGHT TREATMENT

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

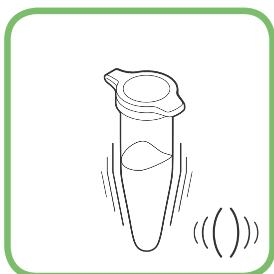
Incubate for **5 min** in the D-Light **with light exposure.**



7. ADD REAGENT D

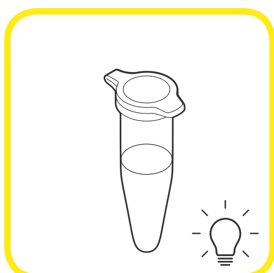
Transfer additional **50 µL** of undiluted Reagent D into the sample.

Note: Avoid extended exposure to light.



8. MIX

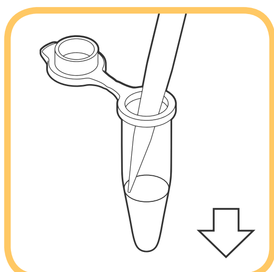
Vortex for **2 sec.**



9. D-LIGHT TREATMENT

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

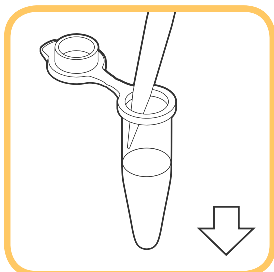
Incubate for **5 min** in the D-Light **with light exposure.**



10. ADD LYSIS BUFFER TO A NEW TUBE

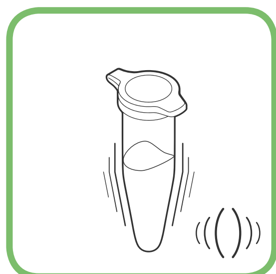
Transfer **200 µL** StarPrep Three Lysis Buffer to a **new** 1.5 mL reaction tube.

Note: Use a magnetic stirrer (low speed) or shake the bottle with lysis buffer immediately before pipetting the buffer.



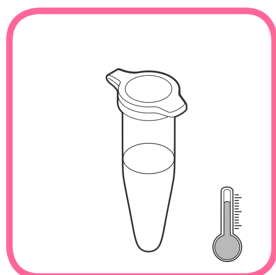
11. ADD SAMPLE

Transfer **15 µL** of the Reagent D treated sample from step 9 into the tube containing lysis buffer.



12. MIX

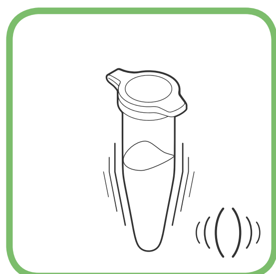
Vortex for **2 sec.**



13. 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**.

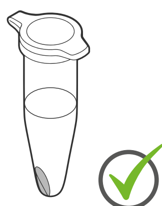


14. MIX

Vortex for **2 sec.**

15. CENTRIFUGE

2 min at 13,000 x g.



SUPERNATANT FOR DETECTION

Use up to 25 µL of extract for the foodproof *Vibrio* Detection LyoKit.

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.

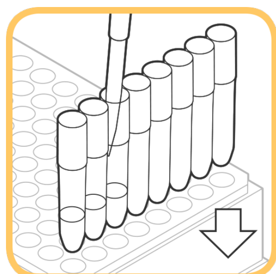
2.3.2 EXTRACTION PROCEDURE B: HIGH THROUGHPUT

The following protocol describes the DNA isolation from 50 µL of enrichment culture using 8-strip tubes and multichannel pipettes. It is recommended when performing more than 16 isolations. Depending on sample matrices, Reagent D treatment (step 3 to 10) may be omitted.

Note: To avoid cross-contamination, always leave one row empty between strip tubes and pipette!

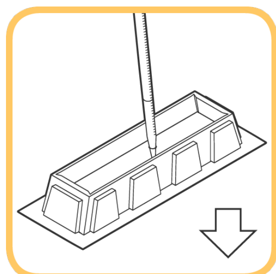
1. SHAKE SAMPLE

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



2. PREPARE SAMPLE

Transfer **100 µL to 400 µL** sample (enrichment culture supernatant) to a first set of 8-tube strips.

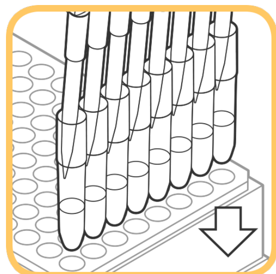


3. PREPARE PREMIX

Mix 350 µL ASPW / APW and 100 µL Reagent D per sample for a total of n samples in a sterile reservoir. Add an additional volume to adjust for pipetting errors:
(n + 1) x 350 µL ASPW / APW + (n + 1) x 100 µL Reagent D.

For example, for five DNA extractions, mix 2.1 mL ASPW / APW (6 x 350) with 600 µL Reagent D (6 x 100).

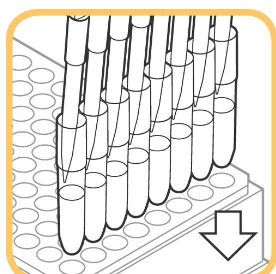
Note: Avoid extended exposure to light.



4. ADD PREMIX TO NEW TUBES

Disperse an aliquot of **450 µL** of the premix to each tube of a **new** strip.

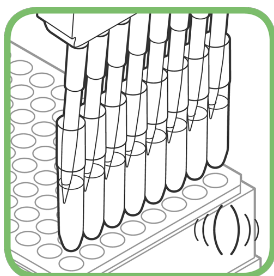
Note: Avoid extended exposure to light.



5. ADD SAMPLE

Transfer **50 µL** from the sample strips (step 2) to the strips filled with premix.

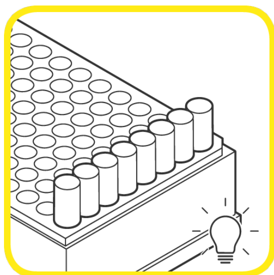
Note: Transfer of all samples should be achieved within 5 minutes.
Avoid extended exposure to light.



6. MIX

Mix **twice** with a multichannel pipette by pipetting up and down with volume set to 400 µL.

Note: Avoid extended exposure to light.

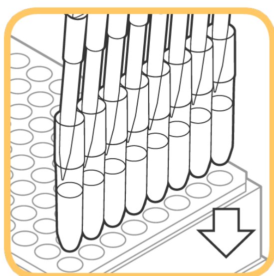


7. D-LIGHT TREATMENT

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

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

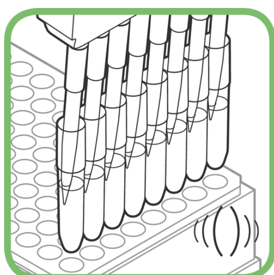
Note: Do not cover with cap strips in this step.



8. ADD REAGENT D

Add additional **50 µL** of undiluted Reagent D into each sample.

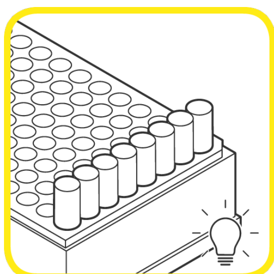
Note: Avoid extended exposure to light.



9. MIX

Mix **twice** with a multichannel pipette by pipetting up and down with volume set to 400 µL.

Note: Avoid extended exposure to light.

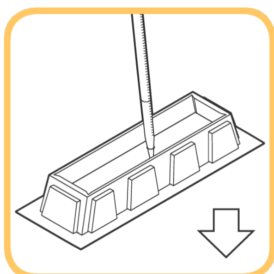


10. D-LIGHT TREATMENT

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

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

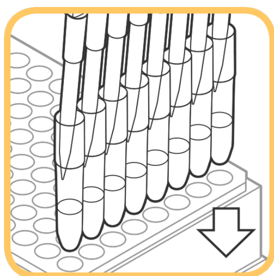
Note: Do not cover with cap strips in this step.



11. PREPARE LYSIS BUFFER

Dilute the needed lysis buffer 2:1 with sterile ddH_2O in a sterile reservoir:
200 µL lysis buffer + 100 µL ddH_2O 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.

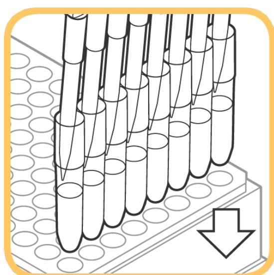


12. ADD LYSIS BUFFER TO NEW STRIPS

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

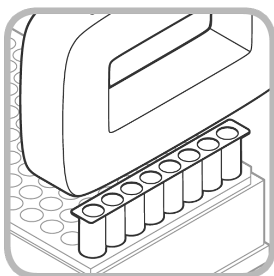
Disperse **300 µL** diluted lysis buffer into new and clean 8-strip tubes.

Note: Avoid extended exposure to light.



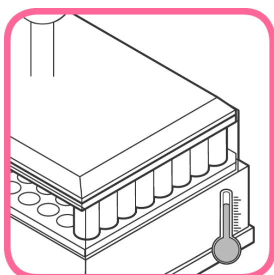
13. ADD SAMPLE

Transfer **20 µL** of the Reagent D treated sample from step 12 to the new strips containing lysis buffer.



14. SEAL TUBES

Seal the tubes tightly with sterile cap strips.



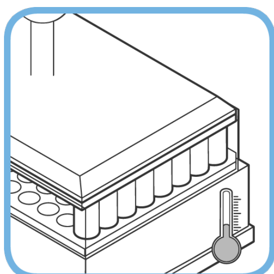
15. INCUBATE

Remove tube rack bottom and install incubation frame.

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

Weigh caps down with the lid weight.

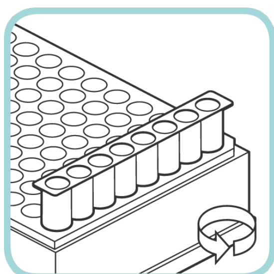
Note: To avoid removing and reinstalling of the rack bottom, it is possible to place tube strips in an empty MicroTube rack (with removed rack bottom).



16. CHILL

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

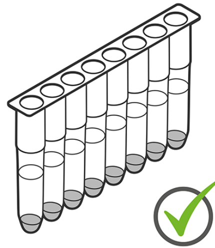
To avoid opening of caps, do not remove the lid weight until the strips are cooled down.



17. 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 (e.g., 9/3). If necessary, centrifugation forces should be calculated according to the manual of the used centrifuge.



SUPERNATANT FOR DETECTION

Use up to 25 µL of extract for the foodproof *Vibrio* Detection 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 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. 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 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. 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 heating block is at correct temperature using 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:

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.

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3.2 Reference Number

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

3.3 Change Index

Version 1, December 2020:
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Version 2, February 2022:
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Revision A, September 2023:
Reformatting, new images and product numbers.
S 400 18 20-1 -> INS-KIT230187-1-REVA

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