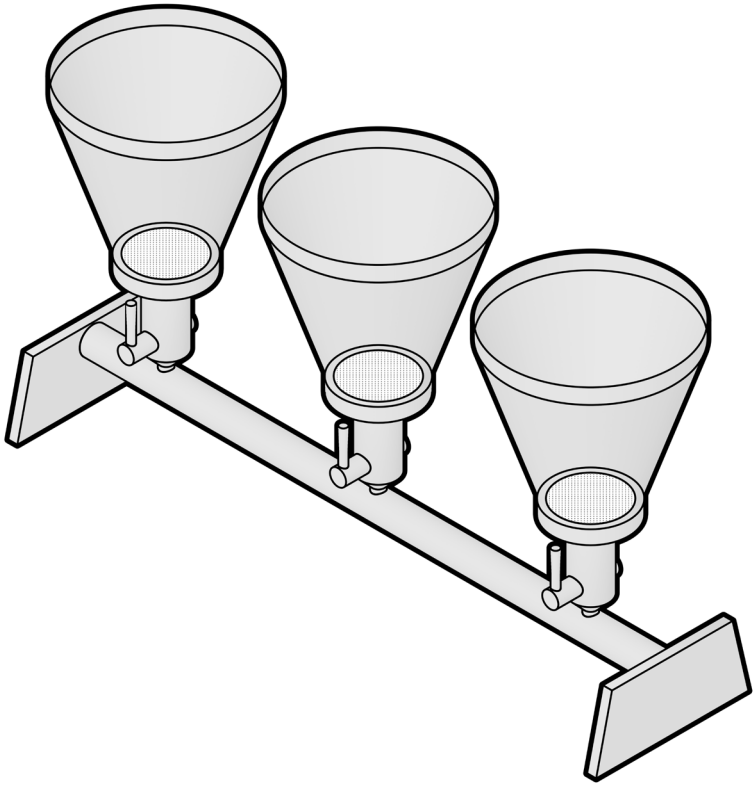


foodproof®

StarPrep Two Kit

Legionella

PRODUCT INSTRUCTIONS

Documentation for the rapid extraction of DNA from *Legionella* for direct use in PCR.

Product No. KIT230177

foodproof®

StarPrep Two Kit

Legionella

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

Product No. KIT230177
42 mL volume

Manual:
Revision A, December 2023

TABLE OF CONTENTS

1. OVERVIEW

The foodproof® StarPrep Two Kit is designed for the rapid preparation of DNA from bacteria or yeast and mold for direct use in PCR. The extracted DNA can be used directly in any PCR application. The StarPrep Two 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 192 reactions.

Storage Conditions

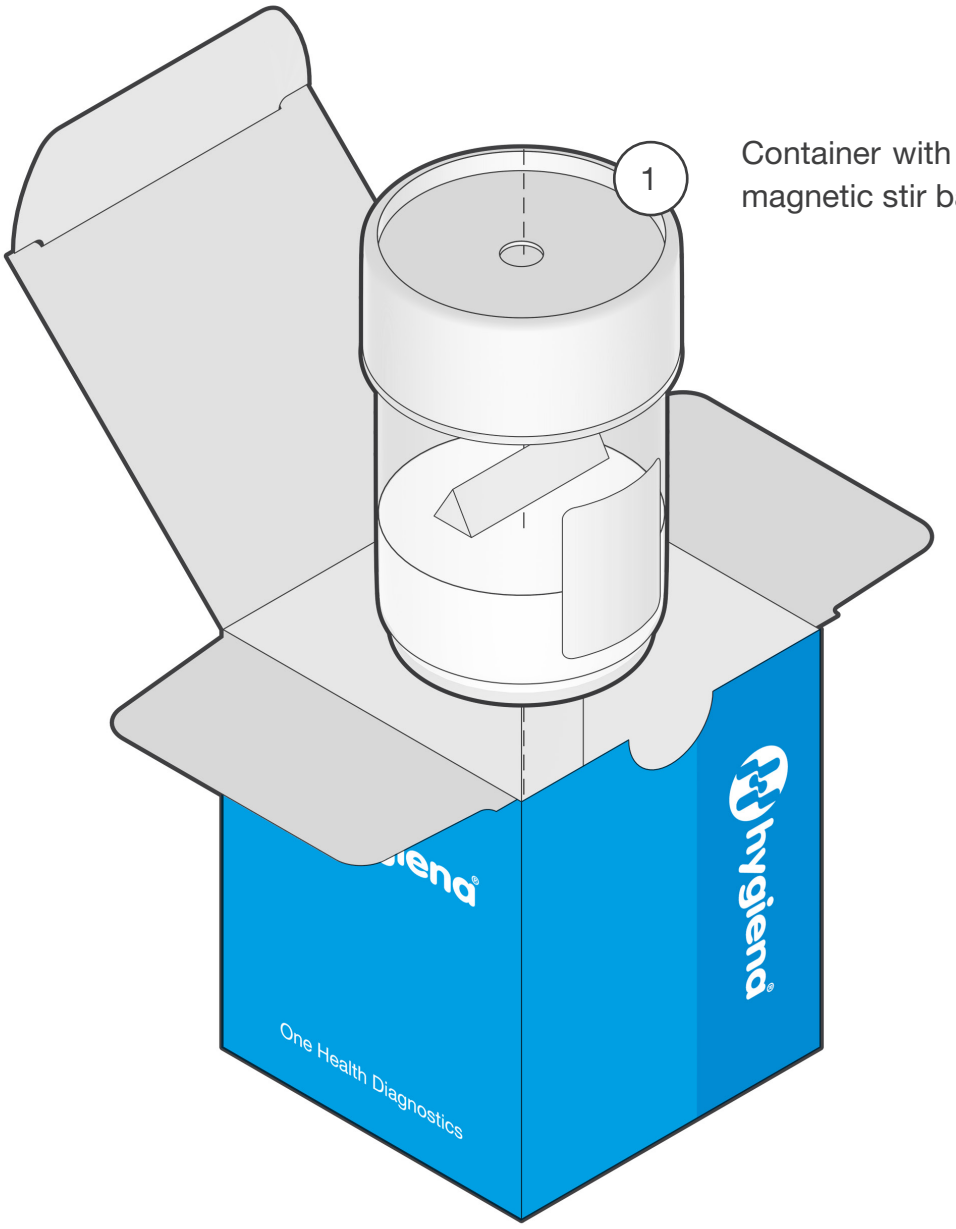
Store at 15 to 25 °C.
The components of the foodproof StarPrep Two Kit are guaranteed to be stable through the expiration date printed on the label.

1.2 Applicability

The lysis buffer is optimized for the preparation of various types of sample material, including enrichment cultures, direct samples and filtered water 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. Strictly adhere to procedures presented in this manual to ensure valid results.

1.3 Kit Contents

A schematic representation of the foodproof StarPrep Two Kit with all its components.



1 Container with 42 mL lysis buffer and magnetic stir bar

2. INSTRUCTIONS

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

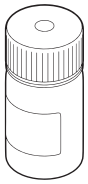
2.1 Required Material

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.

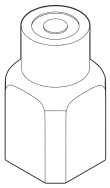
Reagents

☐ **Reagent D**
Product No. KIT230001



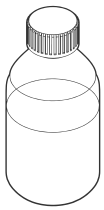
☐ **Rinse Buffer**
Product No. KIT 230012

For Procedures A (2.3.1) and C (2.3.3)



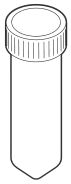
☐ **Diluted Ringer‘s Solution (according to ISO 11731)**
Not provided by Hygiena Diagnostics GmbH

Only for Procedure C (2.3.3)

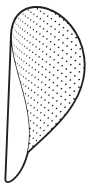


Consumables

☐ **5 mL tubes** (58 mm x 15 mm)

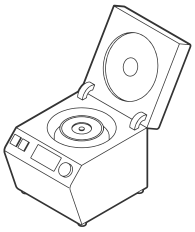


☐ **PES membrane filters**
Pore size: 0.45 µm, diameter: 47 mm
or
Pore size: 0.22 µm, diameter: 47 mm

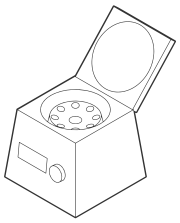


Equipment

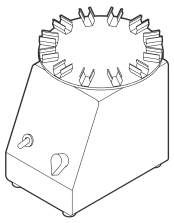
☐ Standard tabletop **microcentrifuge** capable of a 13,000 × g centrifugal force
e.g., Micro Star 17 - VWR



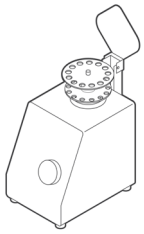
☐ **Centrifuge** suitable for 5 -15 mL tubes and capable of a 1,000 × g centrifugal force
e.g., CAPPRondo - CAPP



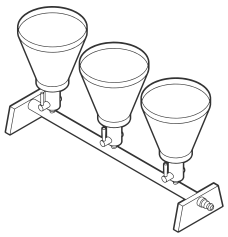
☐ **Vortex mixer**
e.g., Vortex-Genie® 2 - Scientific Industries with "Large Ampule/ Tube Attachment" adapter or Horizontal 15 mL Tube Holder



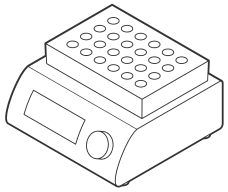
☐ Unit for **mechanical cell disruption** suitable for working with 1.5 mL reaction tubes
e.g., Mortexer™ - Benchmark Scientific
or
Disruptor Genie® - Scientific Industries



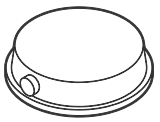
☐ **Filtration unit** suitable for 47 mm filter membranes



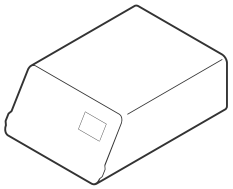
☐ **Heating unit** suitable for 1.5 mL tubes
e.g., AccuBlock™ - Labnet with heating block



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



☐ **D-Light**
Product No. MCH230039

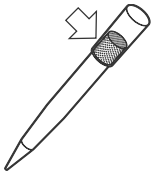


2.2 Precautions and Preparations

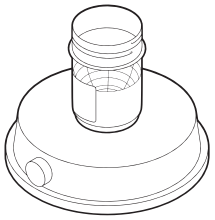
Follow all universal safety precautions governing work with biohazardous materials, e.g., wear lab coats and gloves at all times. Properly dispose of all contaminated materials, decontaminate work surfaces, and use a biosafety cabinet whenever aerosols might be generated.

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

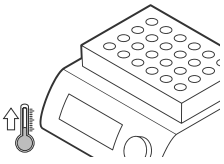
☐ Always use filter tips in order to avoid cross-contamination.



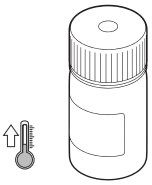
☐ Mix thoroughly while pipetting the buffer for sample preparation.
It is not recommended to use more than 192 reactions. The container must retain some of the reagent. Do not use anymore reagent once the minimum level mark on the container has been reached. The mark indicates the minimal allowed pipetting level while the stirrer is not in use.



☐ 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.



2.3. Workflows

The following procedures describe the DNA isolation from direct or filtered water samples and bacterial colonies.

PROCEDURE A describes the procedure for filtered water samples.

PROCEDURE B can be used for turbid water samples, or other samples, which cannot be filtered.

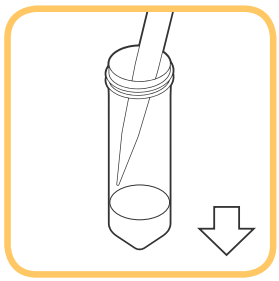
PROCEDURE C enables parallel PCR quantification and enumeration according to ISO 11731:2017.

For colony confirmation, an easy and rapid method can be found in the product instructions for the microproof® Suspension Buffer (KIT230178).

2.3.1 EXTRACTION PROCEDURE A

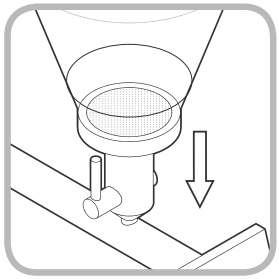
This protocol is intended for quantification of *Legionella* in water samples using 47 mm PES filter disks.

Omit steps 7 and 8 if removal of dead bacteria or residual DNA is not desired.



1. ADD RINSE BUFFER

Transfer **1,000 µL** Rinse Buffer to a 5 mL tube.

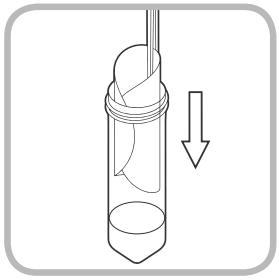


2. FILTER SAMPLES

Use a PES membrane to filter samples in a filtration unit.
Place filter with shiny side upwards

Write down the filtration volume.

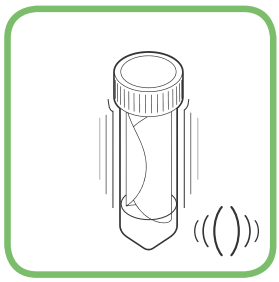
Note: Conduct filtration according to your laboratory's specifications.
Acid or heat treatment of the filter is not necessary for real-time PCR analysis.



3. ADD FILTER TO RINSE BUFFER

Transfer the filter to a 5 mL tube containing the Rinse Buffer
with the top side (filter cake) facing inwards.

Note: Pick up the filter disk at opposing sides using two sterile forceps. Gently roll the membrane into the shape of a cylinder until it fits into the 5 mL tube. Be careful not to crumple the filter disk or touch its top side!
Collecting samples for batch processing is possible at this step. Vials can be kept at 2 to 8 °C for up to 4 hours before proceeding with step 4.

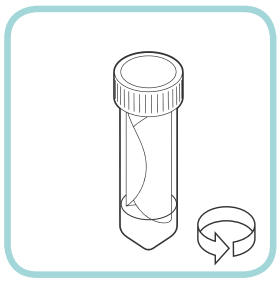


4. VORTEX

Insert tubes into "Large Ampule/Tube Attachment" or
"Horizontal 15 mL Tube Holder" on a Vortex Genie 2.

Vortex for **15 s at full speed**,
then vertically rotate the tubes 180° and **repeat** the process.

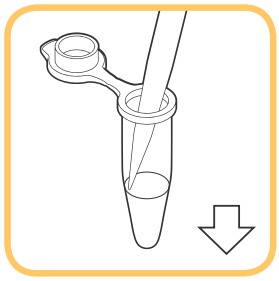
Note: The tubes should be balanced. When processing an odd number of samples, add a 5 mL tube filled with 1 mL distilled water as counterweight.



5. CENTRIFUGE

1 min at 1,000 x g.

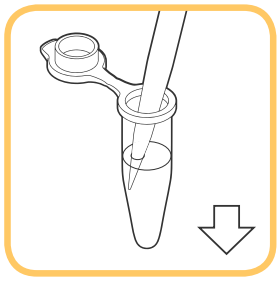
Note: Use a centrifuge suitable for 5 or 15 mL tubes.
If necessary, centrifugation forces should be calculated according to the centrifuge manual used.



6. ADD SAMPLE

Transfer **700 µL** sample (from 5 mL tube) to a transparent 1.5 mL reaction tube.

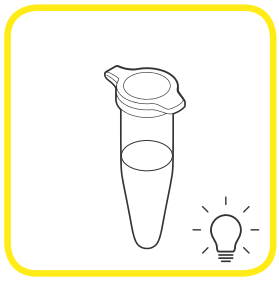
Note: In case recovery of 700 µL cannot be achieved, write down the approx. recovered volume and take this into account when calculating the bacterial load of the sample.



7. ADD REAGENT D

Transfer **300 µL** Reagent D and mix by pipetting up and down 3 to 5 times or by briefly vortexing.

Note: Proceed immediately with the following steps of the protocol. Avoid extended exposure to light. For optimal efficiency, the mixing has to be complete. In case less than 700 µL was transferred in step 6, adjust Reagent D volume accordingly, e.g., for 350 µL sample add 150 µL Reagent D.



8. D-LIGHT TREATMENT

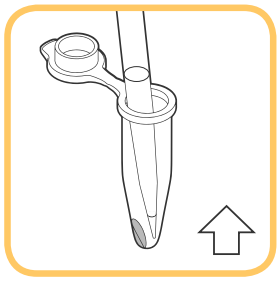
Incubate for **10 min at room temperature** in the D-Light **in the dark.**

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

9. CENTRIFUGE

5 min at 8,000 x g.

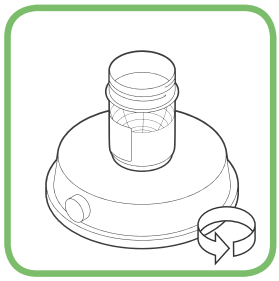
Note: If necessary, centrifugation forces should be calculated according to the centrifuge manual used.



10. 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.



11. PREPARE LYSIS BUFFER

Place closed Lysis Buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

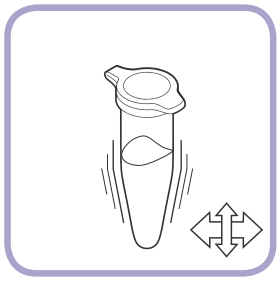
Open the lysis buffer container.

Note: Hold the container while switching on the magnetic stirrer and during pipetting.

12. ADD LYSIS BUFFER

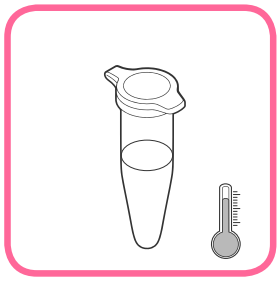
Transfer 150 µL lysis buffer to the sample tube.

Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.



13. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:
Mortexer or Disruptor Genie: 8 min at maximum speed.



14. INCUBATE

5 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**.

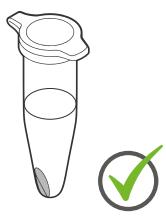
15. MIX

Vortex for 2 s.

16. CENTRIFUGE

1 min at 8,000 to 13,000 x g.

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



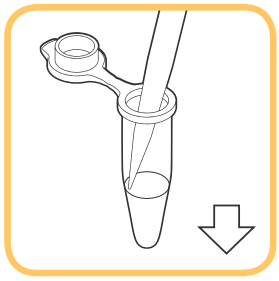
SUPERNATANT FOR DETECTION

Use 25 µL of extract for the microproof *Legionella* Quantification 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 1 min.

2.3.2 EXTRACTION PROCEDURE B

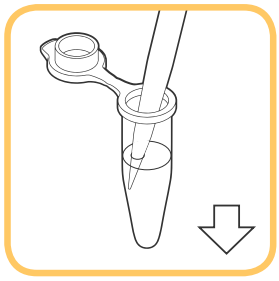
This protocol is intended for direct quantification of *Legionella* in water samples without filtration. Omit steps 2 and 3 if removal of dead bacteria or residual DNA is not desired.



1. ADD SAMPLE

Transfer **700 µL** sample to a transparent 1.5 mL reaction tube.

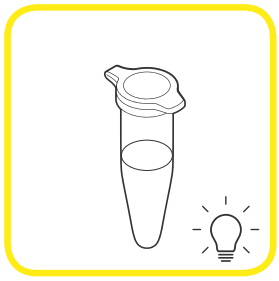
Note: In case of very cloudy samples, only transfer 300 µL or less.
Acid or heat treatment of the sample is not necessary for real-time PCR analysis.



2. ADD REAGENT D

Transfer **300 µL** Reagent D and mix by pipetting up and down 5 to 10 times or by briefly vortexing.

Note: Proceed immediately with the following steps of the protocol.
Avoid extended exposure to light.
For optimal efficiency, the mixing has to be complete.
If the sample volume is reduced, the volume of Reagent D must be adjusted accordingly, e.g., 350 µL sample + 150 µL Reagent D.



3. D-LIGHT TREATMENT

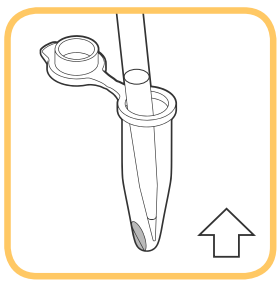
Incubate for **10 min at room temperature** in the D-Light **in the dark.**

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

4. CENTRIFUGE

5 min at 8,000 x g.

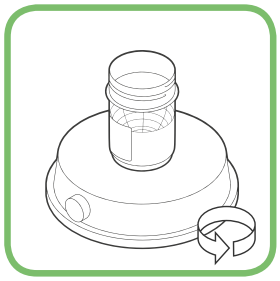
Note: If necessary, centrifugation forces should be calculated according to the cemtrifuge manual used.



5. 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.



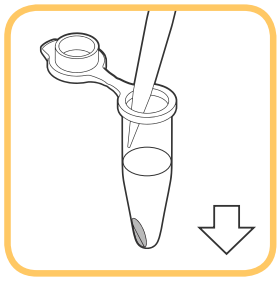
6. PREPARE LYSIS BUFFER

Place closed lysis buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

Open the lysis buffer container.

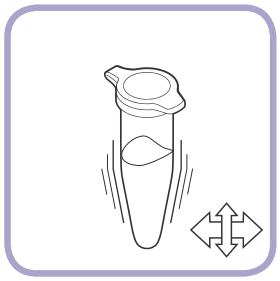
Note: Hold the container while switching on the magnetic stirrer and during pipetting.



7. ADD LYSIS BUFFER

Transfer 150 µL lysis buffer to the sample tube.

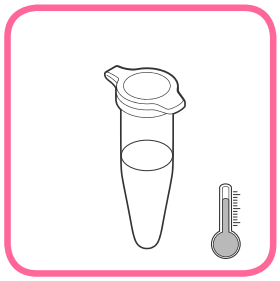
Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.



8. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

Mortexer or Disruptor Genie: 8 min at maximum speed.



9. INCUBATE

5 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**.

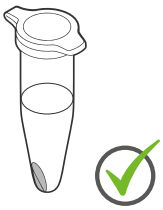
10. MIX

Vortex for 2 s.

11. CENTRIFUGE

1 min at 8,000 to 13,000 x g.

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



SUPERNATANT FOR DETECTION

Use 25 µL of extract for the microproof *Legionella* Quantification LyoKit.

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

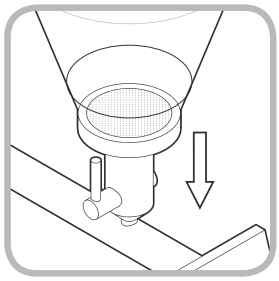
For later analysis, store DNA at -15 to -25 °C.

After thawing, mix briefly by vortexing and centrifuge at 13,000 × g for 1 min.

2.3.3 EXTRACTION PROCEDURE C

This protocol is intended for use of the microproof *Legionella* Quantification LyoKit in conjunction with cultural enumeration according to ISO 11731:2017. This protocol is particularly useful for screening negative samples by PCR prior to plating or comparative enumeration using both methods.

The method conforms to chapter 8.2.3 “Membrane filtration followed by a washing procedure” using 47 mm PES filter disks and the diluents described in “Annex C” of ISO 11731.

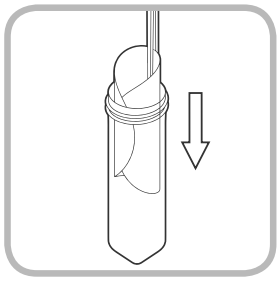


1. FILTER SAMPLES

Use a PES filter to filter samples in a filtration unit.
Place filter with shiny side upwards.

Write down the filtration volume.

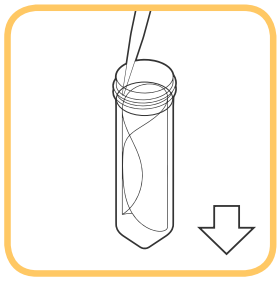
Note: Conduct filtration according to your laboratory’s specifications.
Acid or heat treatment of the filter is not necessary for real-time PCR analysis.



2. FILTER IN TUBE

Transfer the filter to a 5 mL tube with the top side (filter cake) facing inwards.

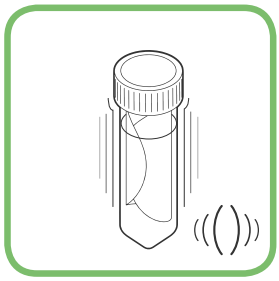
Note: Pick up the filter disk at opposing sides using two sterile forceps. Gently roll the membrane into the shape of a cylinder until it fits into the 5 mL tube. Be careful not to crumple the filter disk or touch its upper side!
Collecting samples for batch processing is possible at this step. Vials can be kept at 2 to 8 °C for up to 4 hours before proceeding with step 4.



3. ADD DILUTED RINGER‘S SOLUTION

Transfer **5 mL** Diluted Ringer’s Solution to a 5 mL tube containing the filter.

Note: Alternatively use PBS [pH 7.5]. Do not use Page’s saline or sterile tap water. These diluents may negatively affect the PCR result.

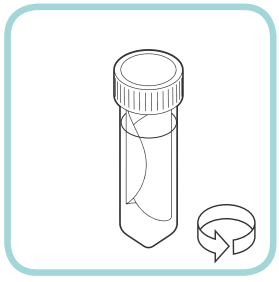


4. VORTEX

Insert tubes into “Large Ampule/Tube Attachment” or
“Horizontal 15 mL Tube Holder” on a Vortex Genie 2.

Vortex for **1 min at full speed**,
then vertically rotate the tubes 180° and **repeat** the process.

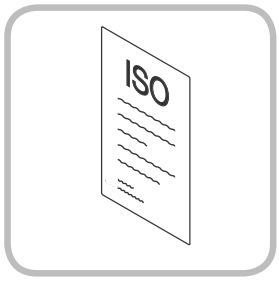
Note: The tubes should be balanced. When processing an odd number of samples, add a 5 mL tube filled with 5 mL distilled water as counterweight.



5. CENTRIFUGE

1 min at 1,000 x g.

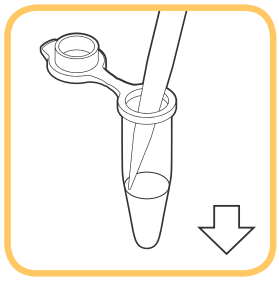
Note: Use a centrifuge suitable for 5 or 15 mL tubes.
If necessary, centrifugation forces should be calculated according to the centrifuge manual used.



6. PROCESS SAMPLE

Use the eluent for culture according to ISO 11731. A part of the eluent may be used for DNA extraction according to the protocol below.

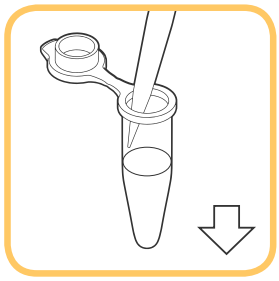
Note: Collecting samples for batch processing is possible at this step. Vials can be kept at 2 to 8 °C for up to 4 hours before proceeding with step 7 or culture.



7. ADD RINSE BUFFER

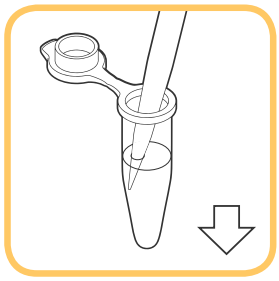
Transfer **500 µL** Rinse Buffer to a 1.5 mL tube.

Note: Alternatively use PBS [pH 7.5]. Do not use Page's saline or sterile tap water. These diluents may negatively affect the PCR result.



8. ADD SAMPLE

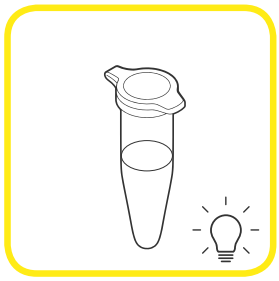
Add **500 µL** eluent from step 6 to the reaction tube containing Rinse Buffer.



9. ADD REAGENT D

Transfer **400 µL** Reagent D and mix by pipetting up and down 3 to 5 times or brief vortexing.

Note: Proceed immediately with the following steps of the protocol. Avoid extended exposure to light. For optimal efficiency, the mixing has to be complete.



10. D-LIGHT TREATMENT

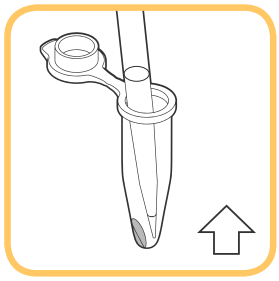
Incubate for **10 min at room temperature** in the D-Light **in the dark.**

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

11. CENTRIFUGE

5 min at 8,000 x g.

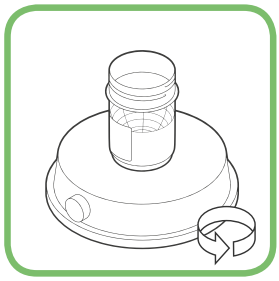
Note: If necessary, centrifugation forces should be calculated according to the centrifuge manual used.



12. 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.



13. PREPARE LYSIS BUFFER

Place closed Lysis Buffer container on the magnetic stirrer.

Continuously mix lysis buffer at 400 rpm on the magnetic stirrer to keep solution homogeneous.

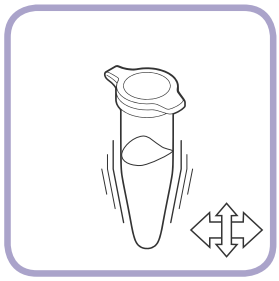
Open the lysis buffer container.

Note: Hold the container while switching on the magnetic stirrer and during pipetting.

14. ADD LYSIS BUFFER

Transfer 150 µL lysis buffer to the sample tube.

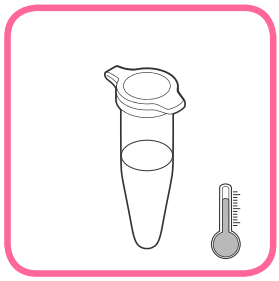
Note: Pipet carefully and vertically along the lysis buffer container wall, approximately 0.5 cm above the bottom.
Use a 1,000 µL filter tip to transfer lysis buffer to the sample.



15. MECHANICAL DISRUPTION

Place tube in a cell disruption unit and perform disruption:

Mortexer or Disruptor Genie: 8 min at maximum speed.



16. INCUBATE

5 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**.

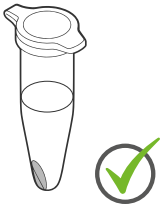
17. MIX

Vortex for 2 s.

18. CENTRIFUGE

1 min at 8,000 to 13,000 x g.

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



SUPERNATANT FOR DETECTION

Use 25 µL of extract for the microproof *Legionella* Quantification 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 1 min.

2.4 Troubleshooting

Problem	Possible Cause	Recommendation
Extract inhibits PCR	Sample contains too many PCR inhibitors.	Repeat DNA extraction with 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.
	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.	Use correct stirring settings. Do not pipet more than 192 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.

2.5 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.

3.2 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers:
S 400 08.1

3.3 Change Index

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

Revision A, December 2023:
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