



Suspension Buffer

Revision A, January 2024

Documentation for the rapid identification of bacterial colonies by PCR.

Product No. KIT230178

40 mL volume

Store the kit at 15 to 25 °C

For microbiological testing purposes.

FOR *IN VITRO* USE ONLY



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1. Overview

The Suspension Buffer is designed for the rapid suspension of bacterial colonies from agar plates, intended to be tested by PCR. The Suspension Buffer eliminates the need for hazardous organic extractions or chaotropic agents. The preparation can be performed in a single tube, minimizing handling steps and exposure to hazardous material. The reduced number of handling steps saves time.

1.1 Applicability

The Suspension Buffer can be used to prepare material from bacterial colonies suitable for any PCR application. DNA is not released during the suspension process but only during the heating steps of the PCR protocol. The extract may therefore contain viable microbial cells which can be regrown for additional analyses such as serotyping or DNA sequencing. Since pathogenic organisms may be present, handle all extracts according to laboratory guidelines for infectious material.

1.2 Kit Contents

Product	Content	Storage
KIT230178	1 container with 40 mL Suspension Buffer	15 to 25 °C

The Suspension Buffer is guaranteed to be stable through the expiration date printed on the label.

2. Instructions

2.1 Required Material

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

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

2.1.1 Reagents (only for LyoKits)

- Internal Amplification Control, Product No. KIT230015

2.1.2 Consumables and equipment

- Sterile reaction tubes
- Sterile inoculation needle, filter tips or toothpick
- Orbital shaker (Vortex)

2.1.3 Consumables (only for high throughput)

- 96-well strip microplates, pureGrade™
- Adhesive seals for 8-well strips, 4titude



2.1.4 Equipment (only for high throughput)

- Electronic 8-channel pipette, EP Xplorer, 0.5 – 10 µL
- Filter tips, 0.1-10 µL, RNase-, DNase-, DNA- and pyrogen-free, box of 96 tips each

or

- Electronic 8-channel pipette VIAFLO, 0.5-12.5 µL
- Filter tips 12.5 µL, sterile, in rack of 384 tips each

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/documents.

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

2.3 Workflows

2.3.1 Procedure for bacterial colonies

This protocol describes the resuspension of a small number of bacterial colonies. This fast protocol requires only a few handling steps. It is recommended to use lyophilized PCR reagents.

Step	Action	Volume
1	<p>ADD BUFFER TO NEW TUBE Transfer 200 µL Suspension Buffer to a 1.5 mL reaction tube.</p> <p>Note: Use only 50 µL in combination with the microproof Hygiene Screening System. For convenient processing of a large number of colonies, use 96-well strip microplates. Remove all strips from the grid and then place an 8-well into every 3rd row. The spacing between strips will help to avoid accidental cross-contamination.</p>	200 µL
2	<p>COLONY TRANSFER Transfer a small portion of a colony with a sterile tool to each tube. Suspend cells by gently stirring with the inoculation tool a couple of times. Avoid rapid motions, which may cause the liquid to spill over.</p> <p>Note: Use a sterile inoculation needle, filter tips or sterile toothpicks.</p>	
3	<p>SUPERNATANT FOR DETECTION Use 5 µL extract for any foodproof®, microproof® or vetproof® PCR kit. For later analysis, store the suspension at 2 to 8 °C for up to several days. The extract may contain viable and potentially infectious cells.</p> <p>Note: In combination with the LyoKits, add 20 µL Internal Amplification Control (Product No. KIT230015) before adding the 5 µL bacterial suspension.</p>	5 µL



2.4 Troubleshooting

Problem	Possible Cause	Recommendation
No internal amplification control signal	Suspension contains too many PCR inhibitors.	Dilute suspension, e.g., 1:10.
	Suspension contains too many target organisms.	Use only 5 µL per reaction. Dilute suspension, e.g., 1:10. Transfer less amount of colony material.
	Internal Amplification Control (Product No. KIT230015) has not been added	Use 20 µL Internal Amplification Control

3. Additional Information

3.1 Quality Control

All products are regularly monitored by our quality control. You can find the certificate of analysis (CofA) on our website. If you would like to carry out your own quality control, you will find the analysis method described in the certificate.

3.2 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. The SDS can be found on our website (*See section 2.2*).

3.3 Warranty and Disclaimer of Liability

“Limited Warranty” and “Disclaimer of Liability”: Hygiena Diagnostics 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 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; 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 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;
- (5) Hygiena Diagnostics 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 reserves the right to replace or allow credit for any modules returned under this warranty.



4. Supplementary Information

4.1 Ordering Information

Hygiena Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.hygiena.com.

4.2 Trademarks

foodproof[®], microproof[®], vetproof[®], ShortPrep[®], RoboPrep[®] and LyoKit[®] are trademarks of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

4.3 Contact and Support

If you have questions or experience problems with this or any other product of Hygiena Diagnostics GmbH, please contact our Technical Support staff (www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. We also want you to contact us if you have suggestions for enhancing our product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to us and the worldwide research community.

4.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: S 400 10

5. Change Index

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New document layout and content.

Revision A, January 2024

Rebranding and new layout.

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