foodproof® *Staphylococcus* Detection LyoKit

Revision A, June 2025

PCR kit for the qualitative detection of coagulase-positive staphylococci plus identification of *Staphylococcus aureus* DNA using real-time PCR instruments.

Product No. KIT230103 (LP), KIT230104 (RP)

Kit for 96 reactions (lyophilized) for a maximum of 94 samples

Store the kit at 2 to 8 °C

FOR IN VITRO USE ONLY

1. Product Overview

1.1 Number of Tests

The kit is designed for 96 reactions, each with a final reaction volume of 25 µL. Up to 94 samples (single sample preparation), plus positive control template and negative control reactions can be analyzed per run.

1.2 Storage and Stability

- Store the kit at 2 to 8 °C through the expiration date printed on the label.
- Once the kit is opened, store the kit components as described in the following Kit Contents table.

1.3 Kit Contents

Component	Label	Contents / Function / Storage
foodproof® *Staphylococcus* Detection LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230103 with white low-profile tubes* • KIT230104 with clear regular profile tubes*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for DNA of coagulase-positive staphylococci, *Staphylococcus aureus* and the Internal Control (IC), as well as Taq DNA Polymerase and Uracil-DNA N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination. • Store at 2 to 8 °C in the aluminum bag (sealed). • **Protect from light and moisture!**
Positive Control	Vial 2 (Purple cap)	• 1 x 300 µL • Contains a stabilized solution of DNA. • For use as a PCR run Quantification Standard. • Store at 2 to 8 °C.
Negative Control	Vial 3 (Colorless cap)	• 2 x 1 mL • Nuclease-free, PCR-grade H_20. • Store at 2 to 8 °C.
Cap Strips	Plastic bag containing 8-cap strips	• 12 × 8 cap strips • For use in real-time PCR after addition of samples.

*Tube profile and instrument capability chart is available by contacting techsupport@hygiena.com .

1.4 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-VIC/HEX and ROX-labeled probes as well as for using low or regular profile strip tubes. In cases where the strip tubes don´t fit the instrument, the samples can be transferred to appropriate PCR vessels (tubes/strips) after resuspension of the lyophilized PCR mix.
- For users of the LightCycler® 480 II (Roche Diagnostics), a color compensation (Color Compensation Set 5, Product No. KIT230011) and a special adapter for PCR strips (Product No. MIS23005) are necessary. For users of the BAX® System Q7 instrument, the HEX calibration kit (Product No. KIT230340) is necessary. Please contact Hygiena® for further information.
- foodproof® StarPrep Two Kit (Product No. KIT230177)

- Nuclease-free, aerosol-resistant pipette tips
- Pipettors
- Centrifuge/Vortex Mix & Spin 32 for 4 x PCR 8-strips (Product No. MCH230064)
  or
- Centrifuge/Vortex CVP-2 for PCR plates (Product No. MCH230036) or 12 x PCR 8-strips with adapter (Product No. MIS230034)

1.5 Applicability Statement

The foodproof *Staphylococcus* Detection LyoKit – 5'Nuclease – is intended for the rapid detection of Coagulase-positive staphylococci plus identification of *Staphylococcus aureus* DNA isolated from enrichment cultures with enrichment conditions e.g. recommended in the ISO methods for *Staphylococcus* (ISO 6888-1, -2, -3:2021) by using the above mentioned sample prep methods of all relevant kinds of foods, feeds and environmental samples that are potentially contaminated with coagulase-positive staphylococci. The foodproof *Staphylococcus* Detection LyoKit is destined for the food and feed industry and for food testing laboratories.

The kit must not be used in diagnostic procedures.

The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM, a VIC/HEX and a ROX detection channel. Kit performance was tested with the following real-time PCR instruments: LightCycler® 480 (Roche Diagnostics), LightCycler® 96 (Roche Diagnostics), Mx3005P®, AriaMx (Agilent Technologies), ABI 7500® Fast (Thermo Fisher Scientific), and CFX96™ (Bio-Rad).

2. Procedure

2.1 Before You Begin

2.1.1 Precautions and Warnings

Detection of coagulase-positive staphylococci plus identification of *Staphylococcus aureus* DNA using the foodproof *Staphylococcus* Detection LyoKit requires DNA amplification by PCR. The kit provides all the reagents required for the PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carry-over-, or cross-contamination:

- Keep the kit components separate from other reagents in the laboratory.
- Use nuclease-free labware (e.g., pipettors, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-preventive pipette tips.
- To avoid carry-over contamination, transfer the required solutions for one experiment into a fresh tube, rather than directly pipetting from stock solutions.
- Physically separate the workplaces for DNA preparation, PCR setup, and PCR to minimize the risk of carry-over contamination. Use a PCR-hood for all pipetting steps.

Note: Keep the foodproof *Staphylococcus* lyophilized PCR Mix away from light and moisture.

2.1.2 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For the preparation of genomic DNA from raw material of plant origin or from food, refer to the corresponding product package inserts of a suitable sample preparation kit (see *Additional Equipment and Reagents Required*). Hygiena Diagnostics provides sample preparation kits suitable for all types of food samples and PPS (see *Additional Equipment and Reagents Required* and www.hygiena.com).

2.1.3 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [foodproof *Staphylococcus* Control Template (vial 2, purple cap)] or with a positive sample preparation control.

2.1.4 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with foodproof *Staphylococcus* Negative Control (vial 3, colorless cap). Include a negative control during sample preparation to monitor reaction purity and cross-contamination. This extraction control can be used as an additional negative control reaction.

2.2 Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (coagulase-positive staphylococci), VIC/HEX (*Staphylococcus aureus*) and ROX (Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR protocol for the foodproof *Staphylococcus* Detection LyoKit. For details on how to program the experimental protocol, see the Instrument Instruction Manual for your real-time PCR-cycler:

Program:

Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2*:	95 °C for 5 minutes
Amplification	**40** cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds

*Fluorescence detection in step 2

For some real-time PCR instruments, the type of the probe quencher as well as the usage of a passive reference dye have to be determined. The foodproof *Staphylococcus* Detection LyoKit contains probes with a nonfluorescent quencher and no passive reference dye.

2.3 PCR Mix Preparation

2.3.1 General Remarks

Proceed as described below to prepare a 25 µl standard reaction. Always wear gloves when handling strips or caps. Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. Note: PCR strips must be stored in the provided aluminum bag with silica gel pads to avoid liquid absorption.

Always wear gloves when handling PCR strips or caps.

Note: The lyophilized material is only stable in the provided aluminum bag with the silica gel pad.

2.3.2 Standard Procedure

1. Remove the needed number of PCR tube strips from the aluminum bag. Use scissors to cut the strips apart. Then, tightly seal the bag and store it at 2 to 8 °C.
2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. Check that the reagent pellets are at the bottom of the tubes. If not, briefly centrifuge or flick the pellets to the bottom before proceeding.
3. Uncap the tube strips cautiously and discard the cap strips.
   Note: To avoid unwanted liquid absorption, only open strips shortly before filling.
4. Pipette 25 µl sample into each PCR-vessel:
   - For the samples of interest, add 25 µL sample DNA (if less, then add PCR-grade H_2O to achieve 25 µL).
   - For the negative control, add 25 µL foodproof *Staphylococcus* Negative Control (vial 3, colorless cap).
   - For the positive control, add 25 µL foodproof *Staphylococcus* Positive Control Template (vial 2, purple cap).

   Note: To reduce the risk of cross-contamination, it is recommended to prepare only one PCR tube strip at a time.
5. Seal the vessels accurately and tightly with the colorless, optical cap strips.
6. Mix thoroughly using a vortex centrifuge.
   Note: Hygiena recommends the vortex centrifuge, Mix & Spin 32, for PCR-strips or the vortex centrifuge, CVP-2, for PCR-plates.
   - For Mix & Spin 32, use the following instrument settings: Spin - 1500 rpm for 5 sec, Vortex - HARD for 20 s, Cycles – 03
   - For CVP-2, use the following instrument settings: Spin - 1000 rpm for 5 s, Vortex - 1200 rpm for 5 sec, Mode - SMS2

   Alternatively, resuspend the pellet manually by cautiously pipetting the sample up and down multiple times during step 4 or by flipping the tube strips after sealing them while pressing down the cap strip.
7. Spin the PCR tube strips for 30 seconds at 150 –200 x g in a suitable centrifuge. If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds.
   Note: Avoid centrifugation at forces exceeding 1,000 x g
8. Place the samples in your PCR cycler and run the program as described above.
   Note: For some PCR instruments, the PCR strips should be placed in a special adapter and balanced in order in the cycler block. For example, two strips can be placed in columns 1 and 12 (see “Additional Equipment and Reagents Required”).

2.4 Procedure – Qualitative Detection

For qualitative detection, compare the results from channels FAM (Coagulase-positive staphylococci), VIC/HEX (*Staphylococcus aureus*) and ROX (Internal Control) for each sample, and interpret the results as described in the table below.

Channel FAM	Channel VIC/HEX	Channel ROX	Result Interpretation
Positive	Positive	Positive or Negative	Positive for *Staphylococcus aureus*
Positive	Negative	Positive or Negative	Positive for Coagulase-positive staphylococci
Negative	Negative	Positive	Negative for Coagulase-positive staphylococci
Negative	Negative	Negative	Invalid

If the amplification in channel FAM or HEX is very weak (Cq ≥ 35), the result may come from a dead cell background. In this case, a prolongation of enrichment and a repetition of the analysis is recommended.

For questions on other PCR instruments, as mentioned above in the "*Applicability Statement,*" please contact our Technical Support team at www.hygiena.com/support or by email at techsupport@hygiena.com.

Note: A prerequisite for the unambiguous discrimination of the targets in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM, HEX and ROX. Please refer to the instruction manual for your real-time PCR cycler for further information.

3. Appendix

3.1 Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	• Set Channel settings to FAM, HEX, ROX.
	Pipetting errors or omitted reagents.	• Check for correct pipetting scheme and reaction setup. • Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	• Check the cycle programs. • Select acquisition mode 'single' at the end of each annealing segment of the PCR program.
No signal increase in channel ROX is observed.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Use the recommended DNA sample preparation kit to purify template DNA. • Dilute samples or pipette a lower amount of sample DNA (e.g., 5 µL instead of 25 µL)
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof *Staphylococcus* lyophilized PCR Mix at 2 to 8 °C, protected from light and moisture as indicated in the Kit Contents Table.
	Low initial amount of target DNA.	• Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Negative control samples are positive.	Carry-over contamination.	• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. • Add positive controls after the sample and negative control reaction vessels have been sealed.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	• Always store the lyophilized PCR mix in the aluminum bag with the silica gel pad • Open the tube strip shortly before filling.
Fluorescence intensity varies.	Insufficient centrifugation of the plate.	• Always centrifuge the plate as described.
	Outer surface of the seal is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the plate.

4. Supplementary Information

4.1 How This Product Works

The foodproof *Staphylococcus* Detection LyoKit provides all necessary reagents and a control template for reliable interpretations of results. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included. A hydrolysis probe was designed to bind specifically to the IC, allowing detection in the ROX channel, whereas the *Staphylococcus aureus* DNA is detected in channel HEX and the coagulase-positive staphylococci DNA in the FAM channel. In case of a negative result due to inhibition of the amplification by the sample DNA of interest, the amplification of the IC is suppressed as well, whereas a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of *Staphylococcus aureus* and coagulase-positive staphylococci in the sample. The foodproof *Staphylococcus* Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of *Staphylococcus aureus* and coagulase-positive staphylococci DNA. The described performance of the kit is guaranteed for use only on the real-time PCR instruments listed above.

4.2 Test Principle

1. Using the kit's sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify fragments of specific sequences for the target species.
2. The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probe due to the 5´-nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5´-end with a reporter fluorophore and at the 3´-end with a quencher.
3. During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal amplicon sequence and is cleaved by the 5’ nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.
5. The PCR instrument measures the emitted fluorescence of the reporter dye.

5.3 Prevention of Carry-over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCRs. This technique relies on the incorporation of deoxyuridine triphosphate (dUTP) during all amplification reactions and the pretreatment of all successive PCR mixtures with the heat-labile UNG. The UNG cleaves DNA at any site where a deoxyuridine residue has been incorporated. The resulting abasic sites are hydrolyzed due to the high temperatures during the initial denaturation step and can no longer serve as PCR templates. The heat-labile UNG is inactivated during the initial denaturation step. Native DNA (e.g., the isolated coagulase-positive staphylococci genomic DNA) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in the foodproof *Staphylococcus* Detection LyoKit, decontamination can be achieved with the provided reagents.

5.4 Product Characteristics

5.4.1 Specificity

Inclusivity/exclusivity of the foodproof *Staphylococcus* Detection LyoKit has been tested with 79 strains comprising of the group of coagulase-positive staphylococci, including 55 *Staphylococcus aureus* strains and more than 75 strains of coagulase-negative staphylococci and other species (mostly from closely related genera). All coagulase-positive staphylococci strains were detected in the FAM channel, all *Staphylococcus aureus* strains were also detected in channel HEX/VIC and no coagulase-negative staphylococci strains were detected in any channel.

5.4.2 Sensitivity

A relative detection limit of 1 to 5 cells per 1g or 10 g sample can be achieved with all relevant kinds of foods. The foodproof *Staphylococcus* Detection LyoKit detects down to 10^3 CFU/mL of coagulase-positive staphylococci enrichment culture (depending on the sample preparation kit used).

5.4.3 Temperature Robustness

The temperature limits of the foodproof *Staphylococcus* Detection LyoKit are the following:

- Denaturation temperature: 95 ± 2.75 °C
- Annealing temperature: 60 ± 2.75 °C

The limits were determined according to Annex C of ISO/DIS 20836:2020.

Real-Time Thermal Cyclers are compatible with the real-time PCR assay when operating within the stated temperature specification limits.

5.4.4 Quality Control

The foodproof *Staphylococcus* Detection LyoKit (LP-KIT230103) is function tested using the LightCycler 480 II System, but can also be run on the BAX System Q7. RP LyoKits are also available for other cyclers and were function tested on appropriate instruments.

5.5 Ordering Information

Hygiena Diagnostics offers a broad range of reagents, kits and services. For a complete overview and more information, visit our website at www.hygiena.com.

5.6 License Notice

NOTICE TO PURCHASER: LIMITED LICENSE

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Email: outlicensing@lifetech.com.

5.7 Trademarks

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5.9 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.

5.10 Reference Number

The original Hygiena Diagnostics GmbH reference number and article number: R 60230-1 (LP) and R 60230- 2 (RP).

6. Change Index

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First version of the product instructions.

Hygiena®
Camarillo, CA
93012 USA

techsupport@hygiena.com

Manufactured by
Hygiena Diagnostics GmbH

Hermannswerder 17

14473 Potsdam
Germany
www.hygiena.com