foodproof® Porcine Detection LyoKit

Revision A, December 2023

PCR kit for the qualitative detection of porcine animals (*Sus scrofa*) by using real-time PCR instruments.

Product No. KIT230115 (LP), KIT230116 (RP)

Kit for 96 reactions (lyophilized) for a maximum of 94 samples plus 2 controls

Store the kit at 2 to 8 °C

FOR *IN VITRO* USE ONLY

Table of Contents

1. Product Overview

1.1 Product Information

The foodproof® Porcine Detection LyoKit was developed to reliably detect DNA of porcine species in raw material, food-, feed-, and some pharmaceutical and cosmetic products with real-time PCR instruments. The foodproof Porcine Detection LyoKit enables a very sensitive analysis by using amplification of multi-copy targets with porcine specific primers and probes. The limit of detection is 0.0001 % or 1 ppm pork in meat products. Because of the high sensitivity of the test, the kit is suitable for analyzing products like gelatin, sweets or other highly processed products. With our Internal Control as well as our Control Template and Negative Control, the reactions monitor the PCR run validity. Our reactions contain two enzymes, Taq DNA Polymerase and Uracil-DNA N-Glycosylase, the latter to prevent carry-over contamination and to reduce false-positive results in your laboratory.

1.2 Number of Tests

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

1.3 Storage and Stability

- Store the kit at 2 °C 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:

1.4 Kit contents

Component	Label	Contents / Function / Storage
foodproof Porcine Detection LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing a 8-tube strip mat • KIT230115 with white low profile (LP) tubes* • KIT230116 with clear regular profile (RP) tubes*	• 96 prefilled reactions (lyophilized) • Ready-to-use PCR mix containing primer and hydrolysis probes specific for porcine DNA (*Sus scrofa),* & the Internal Control (IC) • Internal Control Plasmid • Taq DNA Polymerase • Uracil-DNA N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination • For amplification and detection of porcine animals (*Sus scrofa*) and Internal Control (IC) sequences. • Store at 2 to 8 °C in the aluminum bag (sealed) • **Protect from light and moisture!**
Control Template	Vial 2 (purple cap)	• 1 x 250 µL • Contains a stabilized solution of DNA • For use as a PCR run positive control • Store at 2 to 8 °C
H_2O PCR-grade	Vial 3 (colorless cap)	• 2 x 1 mL • Nuclease-free, PCR-grade H_2O • For use as a PCR run negative control
Cap strips	Plastic bag containing 8-cap strips	• 12 x 8-cap strip • For use in real-time PCR after addition of samples

*Tube profile and instrument compatibility chart is available online: hygiena.com/document-library

1.5 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, and HEX-labeled probes as well as for using low or regular profile 8-strip tubes. In cases where the strip tubes don´t fit in the instrument, the samples must be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.
- Sample Preparation Kit
    - foodproof Sample Preparation Kit III for animal identification (Product No. KIT230174) for manual extraction
    - foodproof Magnetic Preparation Kit III for animal identification (Product No. KIT230182) for automation
    - foodproof StarPrep Two Kit for animal identification (Product No. KIT230177); optional for some matrices
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex centrifuge Multispin MSC-6000 for PCR strips **with** SR-32, Rotor for MSC-3000/6000
  Or Vortex centrifuge CVP-2 for PCR plates

Note: *More detailed information is available from our Technical Support Team (hygiena.com/support).*

For some gelatin products and instant food products, we recommend using the foodproof Sample Preparation Kit III to obtain optimal results. Please ask for our Validation Data Report to review the list of matrices tested and the applicability of Sample Preparation Kit III vs. StarPrep Two Kit for these matrices, respectively.

1.6 Applicability Statement

The foodproof Porcine Detection LyoKit is intended for the rapid detection of porcine animal DNA, including domestic pig and wild boar, from preparations of processed food as well as feed samples and pharmaceutical products.

The foodproof Porcine Detection LyoKit has been developed for real-time PCR instruments with FAM and HEX detection channels.

The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480, LightCycler 96 (Roche Diagnostics), Mx3005P® (Agilent Technologies), ABI 7500 FAST (Applied Biosystems), and PikoReal® 24 (Thermo Scientific). Other real-time PCR instruments may be used; contact us to request additional information.

2. How to use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of DNA from porcine animals using the foodproof Porcine Detection LyoKit requires DNA amplification by PCR. The kit provides all 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., pipettes, 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 Porcine Detection LyoKit 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 preparation of DNA from raw material of animal origin food or pharmaceutical products, refer to the corresponding product package inserts of a suitable sample preparation kit (see *"Additional Equipment and Reagents Required"*).

2.1.3 DNA Extraction

Hygiena Diagnostics GmbH provides sample preparation kits suitable for all kinds of raw material and food samples (see *"Additional Equipment and Reagents Required"*).
For more product information, please see www.hygiena.com.

2.1.4 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 Porcine Detection LyoKit Control Template (vial 2, purple cap)] or with a positive sample preparation control.

2.1.5 Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water (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

2.2.1 Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (for porcine animals) and HEX/VIC (for Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples.

Use the following real-time PCR protocol for the foodproof Porcine Detection LyoKit. For details on how to program the experimental protocol, see the Instrument Operator's Manual of your real-time PCR cycler:

Program for Food, Feed, Cosmetic or Pharmaceutical Samples:	
Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 10 minutes
Amplification	**35** cycles[1]
Step 1:	95 °C for 5 seconds
Step 2[2]:	60 °C for 60 seconds

[1] a) When using the Light Cycler 480, values >30 will not be displayed properly if the amplification program stops at 35 cycles. More detailed information is available from our Technical Support Team at www.hygiena.com/support.

[1] b) When performing automated DNA extraction using the foodproof Magnetic Preparation Kit III, we recommend checking if 35 cycles are appropriate. More detailed information is available from our Technical Support Team at www.hygiena.com/support.

[2] Fluorescence detection in step

Notes:

- For some real-time PCR instruments, the type of the probe quencher as well as the usage of a passive reference dye must be specified. The foodproof Porcine Detection LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For users of the Agilent Mx3005P instrument: Click 'Instrument → Filter Set Gain Settings' to open the Filter Set Gain Settings dialog box to view and modify the gain settings. For FAM and Hex detection, the Filter Set Gain Setting has to be modified to 'x1'.

2.2.2 Preparation of the PCR Mix

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.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or a scalpel to cut the strips apart. Tightly seal the bag afterward and store at the recommended conditions.
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. Decap the tube strips cautiously and discard the cap strips.
   Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips only shortly before filling.
4. Pipet 25 µL sample into each PCR vessel:
   - For the samples of interest, add 25 µL sample DNA (if using less volume, add PCR-grade H_2O to achieve 25 µL).
   - For the negative control, add 25 µL PCR-grade H_2O (vial 3, colorless cap).
   - For the positive control, add 25 µL foodproof Porcine Detection Control Template (vial 2, purple cap).

   Note: To reduce the risk of cross-contamination, we recommended preparing only one PCR tube strip at a time.
5. Seal the vessels accurately and tightly with the colorless cap strips.
6. Mix thoroughly using a vortex centrifuge.
   Note: Hygiena Diagnostics recommends vortex centrifuges Multispin MSC-3000 for PCR strips or vortex centrifuge CVP-2 for PCR plates. Dedicated protocols are available for this centrifuge.
   Note: Alternatively, resuspend the pellet by manual mixing. This may be achieved by cautiously pipetting the sample up and down multiple times during step 4 or flipping the tube strips after sealing while pressing down the cap strip.
7. Spin the PCR tube strips for 30 seconds at 150 – 200 x g in a suitable centrifuge.
   Note: If your centrifuge exceeds 200 x g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1000 x g!
8. Place the samples in your PCR cycler and run the program as described above.
   Note: When using any LightCycler 480 instrument, a special adapter is necessary. For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example two strips can be placed in columns 1 and 12.

2.3 Data Interpretation – Qualitative Detection

The amplification of the proc sequence is analyzed in the fluorescence channel suitable for FAM labeled probes detection. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for HEX.

Compare the results from the FAM channel (Porcine) and HEX channel (Internal Control) for each sample, and interpret the results as described in the table below.

Channel FAM (Porcine)	Channel HEX/VIC (Internal Control)	Result Interpretation
Positive	Positive	Positive for Porcine
Positive	Negative	Positive for Porcine
Negative	Positive	Negative for Porcine
Negative	Negative	Invalid

3. 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/VIC.
	Pipetting errors.	• Check for correct reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	• Check the cycle programs.
No signal increase in channel HEX 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. For optimal performance, use foodproof Sample Preparation Kit III for animal identification (Product No. KIT230174) • Dilute samples or pipet a lower amount of sample DNA (e.g., 5 µL instead of 25 µL and add 20 µL H_2O, and a duplicate 1 µL instead of 25 µL and add 24 µL H_2O).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof Porcine Detection LyoKit PCR Mix at 2 to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	• Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline	Resuspension of lyophilized PCR mix not complete	• Always resuspend lyophilized PCR mix thoroughly.
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 sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Resuspend PCR mix is still in the upper part of the vessel.	• Always centrifuge PCR strips.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the vessels and seal.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	• Store the lyophilized PCR mix always in the aluminum bag with the silica gel pad • Open strip shortly before filling.

4. Additional Information on this Product

4.1 How this Product Works

The foodproof Porcine 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 the IC, allowing detection in the Hex channel, whereas the Porcine-DNA is detected 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 Porcine DNA in the sample. The foodproof Porcine Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of porcine DNA. Primers and probes provide specific detection of Porcine-DNA in food-, cosmetic- and pharmaceutical samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only.

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 pork-specific sequences.
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 sequence of the amplicon 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.
4. The PCR instrument measures the emitted fluorescence of the reporter dye.

4.3 Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCR reactions. 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 plant 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 Porcine Detection LyoKit, decontamination can be achieved with the provided reagents.

4.4 Background Information

For some religions, specific animal species are not allowed to be eaten. For instance, the consumption of food products derived from porcine sources is strictly prohibited in Islamic countries.

In parallel, the EU set up a food safety policy, Regulation 178/2002, laying down the general principles and requirements of food law. It requires every food and feed business in Europe and those bringing food/feed into Europe to have a traceability and recall system in place. Traceability is the ability to track any food, feed, food-producing animal or substance that will be used for consumption through all stages of production, processing and distribution.

This product can be used to identify false labeling of food products.

4.5 Product Characteristics

Specificity	The primers and hydrolysis probes (5' nuclease probes) provided in the lyophilized mix are sequence-specific for porcine animals and the Internal Control, respectively. Assay specificity was proven by testing 45 different animal, microorganism and plant species.
Sensitivity	The limit of detection: 0.1 *Sus scrofa* genome equivalent as well as 1 ppm pork spiked in minced meat.
Matrix	Kit was validated with meat products (i.e., lasagna, tomato sauce, sausages, minced meat), instant food, pastries, crisps, feed, cookies, sweets, gelatin products, sunscreen, hand cream. tablets,
Robustness	Reproducibility of Cp values was successfully tested with different real-time PCR instruments, including Roche LightCycler® 480 II, Agilent Mx3005p, Applied Biosystems® 7500 FAST, Thermo Scientific PikoReal, and Bio-Rad iQ™5 Cycler.

4.6 Quality Control

The foodproof Porcine Detection LyoKit (low profile) is function tested using the LightCycler 480 System or (for the regular profile) using the Agilent Mx3005p.

5. Supplementary Information

5.1 Ordering Information

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

5.2 License Notice

The purchase price of this product includes limited, nontransferable rights under US Patent No. 7,687,247 owned by Life Technologies Corporation to use only this amount of the product to practice the claims in said patent solely for activities of the purchaser for bioburden testing, environmental testing, food testing, or testing for genetically modified organisms (GMO) in accordance with the instructions for use accompanying this product. No other rights are conveyed, including no right to use this product for *in vitro* diagnostic, therapeutic, or prophylactic purposes. Further information on purchasing licenses under the above patent may be obtained by contacting the Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008.
Email: outlicensing@lifetech.com.

5.3 Trademarks

foodproof® is a registered trademark of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

5.4 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.

5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article numbers:

R 602 43-1 (KIT230115) and R 602 43-2 (KIT230116).

6. Change Index

Version 1, October 2015
First version of the package insert.

Version 2, December 2016
2.2 Preperation of the PCR Mix updated.

Version 3, March 2017
License Notice changed.
Introduction of vortex centrifuges into the PCR Setup Procedure.

Revision A, December 2023:
Rebranding and new layout.
R 602 43 20 -> INS-KIT230115-16-RevA

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