foodproof® Plant Detection LyoKit

Revision A, March 2024

PCR kit for the qualitative detection of plant DNA using real-time PCR instruments.

Product No. KIT230090 (LP)

Product No. KIT230091 (RP)

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

Store the kit at 2 °C to 8 °C

FOR *IN VITRO* USE ONLY

Table of Contents

1. What This Product Does

1.1 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 positive 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 components as described in the following contents table:

1.3 Kit Contents

Component	Label	Contents / Function / Storage
foodproof® Plant Detection LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230090 with white low-profile (LP) tubes* • KIT230091 with clear regular profile (RP) tubes*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for native plant DNA 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. • For amplification and detection of native plant DNA and Internal Control (IC) sequences. • Store at 2 to 8 °C in the aluminum bag (sealed and containing silica gel pads). • **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: www.hygiena.com/documents

1.4 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, HEX- and ROX-labeled probes as well as for using low or regular profile strip tubes. In case the strip tubes don´t fit the instrument, the samples should be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.
- Sample Preparation Kit
  - foodproof Sample Preparation Kit III (Product No. KIT230174)
  - foodproof Magnetic Preparation Kit II (Product No. KIT230181)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettors

and optionally

- Vortex centrifuge Multispin MSC-6000 for PCR strips **with**

SR-32, Rotor for MSC-3000/6000 **or**
- Vortex centrifuge CVP-2 for PCR plates

1.5 Applicability Statement

The foodproof Plant Detection LyoKit (5'Nuclease) is intended for the rapid detection of plant DNA from preparations of raw material and processed food as well as feed and seed samples.

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 and a HEX detection channel. 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).

***Note**:* A color compensation (Color Compensation Set 3, Product No. KIT230005) is necessary and will be supplied by Hygiena® Diagnostics for users of the LC 480 Systems I and II. Please contact Hygiena Diagnostics for further information.

2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of DNA from plant origin using the foodproof Plant 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., pipettors, pipette tips, reaction vials).
- Wear gloves when performing the assay.
- To avoid cross-contamination of samples and reagents, use fresh aerosol-barrier 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.

Keep the foodproof Plant Detection 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 preparation of genomic DNA from various sample enrichments, 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 provides sample preparation kits suitable for all types of food and environmental samples (see *"Additional Equipment and Reagents Required"*). For more product information, please refer to 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 (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 H_2O (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 Procedure

2.2.1 Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (for plant DNA) and HEX (for Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR protocol for the foodproof Plant 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 the Roche LightCycler 480, LightCycler 96, and ABI 7500 FAST:

Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 10 minutes
Amplification	**50** cycles
Step 1:	95 °C for 5 seconds
Step 2*:	60 °C for 60 seconds

Program for other real-time PCR instruments:

Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 10 minutes
Amplification	**50** cycles
Step 1:	95 °C for 15 seconds
Step 2*:	60 °C for 60 seconds

* Fluorescence detection in step 2

Notes:

- For some real-time PCR instruments, the type of the probe quencher as well as the usage of a passive reference dye has to be specified. The foodproof Plant 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 in which the gain settings may be viewed and modified. For FAM and HEX, the Filter Set Gain Setting must be modified to 'x4'. For ROX and Cy5, the Filter Set Gain Setting must 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 under 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 (for less volume, add PCR-grade water to achieve a total volume of 25 µL).
   - For the negative control, add 25 µL PCR-grade H_2O (vial 3, colorless cap).
   - For the positive control, add 25 µL Plant 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 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 manually mixing by cautiously pipetting the sample up and down multiple times during step 4 or by 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 1,000 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

The amplification of the plant DNA is analyzed in the fluorescence channel suitable for FAM-labeled probe detection. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for HEX.

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

Channel FAM (Plant)	Channel HEX (Internal Control)	Result Interpretation
Positive	Positive or Negative	Positive for plant DNA
Negative	Positive	Negative for plant DNA
Negative	Negative	Invalid

Note: A prerequisite for the unambiguous discrimination of plant DNA and Internal Control DNA amplification in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM and HEX. Please refer to the operation manual for your real-time PCR cycler for further information.

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 or ROX.
	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 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 pipet 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 Plant Detection Lyophilized 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.	• 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. • 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. Resuspended PCR mix is still in the upper part of the vessel.	• Centrifuge PCR strips.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	• Wear gloves when handling the vessels and seal.
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 strip immediately before filling.

4. Additional Information on this Product

4.1 How this Product Works

The foodproof Plant 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 plant 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 plant DNA in the sample. The foodproof Plant Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of plant DNA. Primers and probes provide specific detection of plant DNA in food samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only. The assays are according to ISO 21569 and to German Food Law § 64 LFGB for the detection of genetically modified DNA sequences [1].

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 plant 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 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.
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 Plant Detection LyoKit, decontamination can be achieved with the provided reagents.

4.4 References

1. International Organization of Standardization. ISO 21569:2013, Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods, 2013-08.

4.5 Quality Control

The foodproof Plant Detection LyoKit is function tested using the LightCycler 480 System.

5. Supplementary Information

5.1 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.

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

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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 numbers and original Hygiena Diagnostics GmbH article numbers:
R 602 21-2 (KIT230090, LP) and R 602 21-2 (KIT230091, RP).

6. Change Index

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

Version 2, March 2017
License Notice changed.
Introduction of vortex centrifuges into the PCR Setup Procedure.

Revision A, March 2024
Rebranding and new layout.
R 602 21 20 -> INS-KIT-230090-91-RevA

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