foodproof® GMO Screening 2 LyoKit

Revision A, September 2023

PCR kit for the qualitative detection of genetically modified organisms (GMO) by screening for bar, P-35S-pat, CTP2-CP4-EPSPS, P-NOS-nptII, and P-35S-nptII using real-time PCR instruments.

Product No. KIT230086 (LP)

Product No. KIT230087 (RP)

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

Store the kit at 2 to 8 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

Table of Contents

1. Product Overview

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 of Kit/Components

- 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.

Component	Label	Contents / Function / Storage
foodproof® GMO Screening 1 LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • KIT230086 (LP) with white low profile tubes* • KIT230087 (RP) with clear regular profile tubes*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for the *bar* gene of *Streptomyces hygroscopicus*, the P-35S-pat construct-specific sequence, the CTP2-CP4-EPSPS construct-specific sequence, the P-NOS-nptII construct-specific sequence, the P-35S-nptII construct-specific sequence 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 *bar*, P-35S-pat, CTP2-CP4-EPSPS and P-NOS-nptII or P-35S-nptII. • Store at 2 °C 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.

1.3 Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and Cy5-labeled probes as well as for using low or regular profile strip tubes. In cases the strip tubes don´t fit for the instrument the samples have to be transferred after resuspension of the lyophilized PCR mix to appropriate PCR vessels.
- Sample Preparation Kit
  foodproof Sample Preparation Kit III (Product No. KIT230174) or
  foodproof Magnetic Preparation Kit III (Product No. KIT230182)
- 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

1.4 Applicability Statement

The foodproof GMO Screening 2 LyoKit is intended for the rapid detection of one or more of the five inserted primary control sequences (bar, P-35S-pat, CTP2-CP4-EPSPS, P-NOS-nptII or P-35S-nptII) in genetically modified plants 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, a HEX, a ROX and a Cy5 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), AriaMx® (Agilent Technologies), and PikoReal® 24 (Thermo Scientific).

Note: A color compensation (Color Compensation Set 3; Product No. KIT230005) is necessary and can be purchased from Hygiena for users of the LC 480 Systems I and II. Please contact Hygiena for further information.

2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of DNA from genetically modified organisms (GMO) using the foodproof GMO Screening 2 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.

Keep the foodproof GMO Screening 2 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 GmbH provides sample preparation kits suitable for all kinds 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 [foodproof GMO Screening 2 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 H_2O PCR-grade (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 bar), HEX (for P-35S-pat), ROX (for CTP2-CP4-EPSPS) and Cy5 (for P-NOS-nptII and P-35S-nptII) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR-protocol for the foodproof GMO Screening 2 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, AriaMx® and ABI 7500 FAST:		**Program for other real-time PCR instruments:**	
Pre-incubation	**1** cycle	Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes	Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 10 minutes	Step 2:	95 °C for 10 minutes
Amplification	**50** cycles	Amplification	**50** cycles
Step 1:	95 °C for 5 seconds	Step 1:	95 °C for 15 seconds
Step 2*:	60 °C for 60 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 GMO Screening 2 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, the Filter Set Gain Setting has to be modified to 'x1'.

2.3 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: The PCR strips must be stored in the provided aluminum bag with the silica gel pads to avoid liquid absorption.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or scalpel to cut the strips apart. Tightly seal the bag afterwards and store away 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. Uncap 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 GMO Screening 2 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 GmbH 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 g in a suitable centrifuge.

Note: If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1,000 g!

8. Place the samples in your PCR cycler and run the program as described above.

Note: For using any LightCycler 480 instrument, a special adapter (Product No. MIS230005) 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 column 1 and 12.

2.4 Data Interpretation

The amplification of the *bar* gene sequence is analyzed in the fluorescence channel suitable for FAM labeled probes detection. The amplification of the P-35S-pat sequence is analyzed in the fluorescence channel suitable for the detection of HEX labeled and the amplification of the CTP2-CP4-EPSPS sequence is analyzed in the fluorescence channel suitable for the detection of ROX labeled probes. The specific amplification of the P-NOS-nptII or P-35S-nptII sequence is analyzed in the fluorescence channel suitable for Cy5.

Compare the results from channel FAM (bar), channel HEX (P-35S-pat), channel ROX (CTP2-CP4-EPSPS) and channel Cy5 (P-NOS-nptII or P-35S-nptII) for each sample, and interpret the results as described in the table below.

Channel FAM (bar)	Channel HEX (P-35S-pat)	Channel ROX (CTP2-CP4-EPSPS)	Channel Cy5 (P-NOS-nptII or P-35S-nptII)	Result Interpretation
Positive	Positive	Positive	Positive	Positive for bar, P-35S-pat, CTP2-CP4-EPSPS and P-NOS-nptII or P-35S-nptII
Positive	Positive	Positive	Negative	Positive for bar, P-35S-pat and CTP2-CP4-EPSPS
Positive	Positive	Negative	Negative	Positive for bar and P-35S-pat
Positive	Negative	Negative	Negative	Positive for bar
Negative	Positive	Negative	Negative	Positive for P-35S-pat
Negative	Negative	Positive	Negative	Positive for CTP2-CP4-EPSPS
Negative	Negative	Negative	Positive	Positive for P-NOS-nptII or P-35S-nptII
Negative	Positive	Positive	Negative	Positive for P-35S-pat and CTP2-CP4-EPSPS
Negative	Negative	Positive	Positive	Positive for CTP2-CP4-EPSPS and P-NOS-nptII or P-35S-nptII
Positive	Negative	Negative	Positive	Positive for bar and P-NOS-nptII or P-35S-nptII
Negative	Negative	Negative	Negative	Negative for bar, P-35S-pat, CTP2-CP4-EPSPS and P-NOS-nptII or P-35S-nptII

Note: A prerequisite for the unambiguous discrimination of of bar, P-35S-pat, CTP2-CP4-EPSPS and P-NOS-nptII or P-35S-nptII in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM, HEX, ROX and Cy5. Please refer to the operation manual of 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, ROX or Cy5.
	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.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof GMO Screening 2 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	• 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 GMO Screening 2 LyoKit provides all necessary reagents and a control template for reliable interpretations of results. Hydrolysis probes were designed to bind and detect specifically the GMO-DNA in the FAM (bar), HEX (P-35S-pat), ROX (CTP2-CP4-EPSPS) and Cy5 channel (P-NOS-nptII and P-35S-nptII). The foodproof GMO Screening 2 LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of GMO-DNA. Primers and probes provide specific detection of GMO-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, 2, 3].

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 the *bar* gene, the P-35S-pat construct, the CTP2-CP4-EPSPS construct, the P-NOS-nptII construct and the P-35S-nptII construct 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’s. 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 *Cronobacter* 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 GMO Screening 2 LyoKit, decontamination can be achieved with the provided reagents.

4.4 Background Information

In order to improve product quality, agronomic traits, as well as develop resistance to pests, genetic modification of agriculture crops has become a predominant activity of research departments in the agricultural industry. Due to the ongoing debate surrounding food containing genetically modified organisms (GMOs), and consumer requests for unambiguous labeling of genetically modified foods, various countries established, or are currently in the process of establishing, establishing, regulatory frameworks dedicated to GMOs (e.g., Europe [4]). In order to take such frameworks into account, reliable methods for GMO screening in food products are required. The foodproof GMO Screening 2 LyoKit provides a simple and rapid molecular method for the simultaneous detection of the bar, P-35S-pat, CTP2-CP4-EPSPS, P-NOS-nptII and P-35S-nptII sequences in DNA preparations from raw material and food samples. The phosphinothricin acetyl transferase gene from *Streptomyces hygroscopicus* (*bar*), the DNA transition sequence between the 35S-promoter of the cauliflower mosaic virus and the modified phosphinothricin acetyl transferase gene from *Streptomyces viridochromogenes* (*pat*), the transition sequence

from CTP2 (chloroplast transit peptide signal sequence from *Arabidopsis thaliana*) to the herbicide tolerance gene *cp4-epsps* (5-enolpyruvylshikimate-3-phosphate synthase gene from *Agrobacterium tumefaciens* strain CP4), the DNA transition sequence between the promoter region of the nopaline synthase gene of *Agrobacterium tumefaciens* (*P-nos*) or the 35S-promoter of the cauliflower mosaic virus (P-35S) and the neomycin-phosphotransferase gene (*nptII*) from the Tn5 transposon of *Escherichia coli* K12 are five sequences commonly found in genetically modified plants.

5. References

1. International Organization of Standardization. ISO 21569, Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods, 2013-08.
2. International Organization of Standardization. ISO/TS 21569-3 Horizontal methods for molecular biomarker analysis - Methods of analysis for the detection of genetically modified organisms and derived products - Part 3: Construct-specific real-time PCR method for detection of P35S-pat-sequence for screening genetically modified organisms, 2015-2.
3. Reiting, R. Real-time PCR methods for the detection of DNA constructs with the nptII gene for the detection of genetically modified plants in food, feed and seed. *J. Verbr. Lebensm*., 5, 2010, pp. 377-39.
4. Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.

6. Supplementary Information

6.1 Quality Control

The foodproof GMO Screening 2 LyoKit is function tested using the LightCycler 480 System.

6.2 Ordering Information

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

6.3 License Notice

The purchase price of this product includes limited, nontransferable rights under U.S. 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.

6.4 Trademarks

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

6.5 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.

6.6 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 602 18

7. 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, September 2023:
Rebranding and new layout.
R 602 18 20 -> INS-KIT230086-87-REVA

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