



foodproof® GMO Soya Identification 1 LyoKit

(DAS-44406-6, MON87701, MON87708, MON87769)

Revision A, December 2023

PCR kit for the qualitative detection of the genetically modified (GM) soya events DAS-44406-6, MON87701, MON87708, and MON87769 DNA using real-time PCR instruments.

Product No. KIT230095 (LP), KIT230096 (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



Table of Contents

- 1. What This Product Does.....3**
 - 1.1 Number of Tests3
 - 1.2 Storage and Stability3
 - 1.3 Kit contents.....3
 - 1.4 Additional Equipment and Reagents Required.....3
 - 1.5 Applicability Statement4
- 2. How to Use this Product4**
 - 2.1 Before You Begin4
 - 2.1.1 Precautions4
 - 2.1.2 Sample Material4
 - 2.1.3 DNA Extraction4
 - 2.1.4 Positive Control5
 - 2.1.5 Negative Control5
 - 2.2 Procedure5
 - 2.2.1 Program Setup5
 - 2.2.2 Preparation of the PCR Mix.....6
 - 2.3 Data Interpretation6
- 3. Troubleshooting8**
- 4. Additional Information on this Product9**
 - 4.1 How this Product Works9
 - 4.2 Test Principle9
 - 4.3 Prevention of Carry-Over Contamination9
 - 4.4 Background Information.....9
 - 4.5 References10
 - 4.6 Quality Control10
- 5. Supplementary Information10**
 - 5.1 Ordering Information.....10
 - 5.2 License Notice.....10
 - 5.3 Trademarks.....10
 - 5.4 Contact and Support.....10
 - 5.4 Reference Number.....11
- 6. Change Index11**



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 kit components as described in the following kit contents table:

1.3 Kit contents

Component	Label	Contents / Function / Storage
foodproof® GMO Soya Identification 1 LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing a 8-tube strip mat <ul style="list-style-type: none"> • KIT230095 (LP) with white low-profile tubes* • KIT230096 (RP) with clear regular profile tubes* 	<ul style="list-style-type: none"> • 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for the GM soya events DAS-44406-6, MON87701, MON87708, and MON87769 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 DAS-44406-6, MON87701, MON87708 and MON87769 specific sequences. • Store at 2 to 8 °C in the aluminum bag (sealed). • Protect from light and moisture!
Control Template	Vial 2 (purple cap)	<ul style="list-style-type: none"> • 1 x 300 µL • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.
H ₂ O PCR-grade	Vial 3 (colorless cap)	<ul style="list-style-type: none"> • 2 x 1 mL • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	<ul style="list-style-type: none"> • 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-, ROX- and Cy5-labeled probes as well as for using low- or regular-profile strip tubes. In cases the strip tubes don't fit 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 (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



1.5 Applicability Statement

The foodproof GMO Soya Identification 1 LyoKit is designed to detect the event-specific genes of four GM soya events (DAS-44406-6, MON87701, MON87708, and MON87769) from preparations of raw material and processed food as well as feed and seed samples.

The kit is intended for food testing purposes only.

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[®], AriaMx[®] (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 us](#) 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 Soya Identification 1 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 runs to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.

Keep the foodproof GMO Soya Identification 1 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 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”*).

2.1.3 DNA Extraction

Hygiena Diagnostics 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 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 Soya Identification 1 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 Procedure

2.2.1 Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (DAS-44406-6), HEX (MON87701), ROX (MON87708), and Cy5 (MON87769) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR protocol for the foodproof GMO Soya Identification 1 LyoKit. For details on how to program the experimental protocol, see the Instrument Operator’s Manual of your real-time PCR cycler:

<u>Pre-incubation</u>	1 cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 10 minutes
<u>Amplification</u>	50 cycles
Step 1:	95 °C for 5 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 use of a passive reference dye has to be specified. The foodproof GMO Soya Identification 1 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 has to be modified to ‘x4’.



2.2.2 Preparation of the PCR Mix

Proceed as described below to prepare a 25 μ L 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 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 it 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 μ L sample into each PCR vessel:
 - For the samples of interest, add 25 μ L sample DNA (if using less volume, add PCR-grade water to achieve 25 μ L).
 - For the negative control, add 25 μ L PCR-grade water (vial 3, colorless cap).
 - For the positive control, add 25 μ L foodproof GMO Soya Identification 1 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 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

The amplification of the DAS-44406-6 sequence is analyzed in the fluorescence channel suitable for FAM-labeled probe detection. The amplification of the MON87701 sequence is analyzed in the fluorescence channel suitable for the detection of HEX-labeled probes, the amplification of the MON87708 sequence is analyzed in the fluorescence channel suitable for the detection of ROX-labeled probes, and the amplification of the MON87769 sequence is analyzed in the fluorescence channel suitable for the detection of Cy5-labeled probes.

Compare the results from channel FAM, channel HEX, channel ROX, and channel Cy5 for each sample, and interpret the results as described in the table below.



Channel FAM (DAS-44406-6)	Channel HEX (MON87701)	Channel ROX (MON87708)	Channel Cy5 (MON87769)	Result Interpretation
✓	✓	✓	✓	Positive for DAS-44406-6, MON87701, MON87708 and MON87769
✓	✗	✗	✗	Positive for DAS-44406-6
✗	✓	✗	✗	Positive for MON87701
✗	✗	✓	✗	Positive for MON87708
✗	✗	✗	✓	Positive for MON87769
✓	✓	✗	✗	Positive for DAS-44406-6 and MON87701
✓	✗	✓	✗	Positive for DAS-44406-6 and MON87708
✓	✗	✗	✓	Positive for DAS-44406-6 and MON87769
✗	✓	✓	✗	Positive for MON87701 and MON87708
✗	✓	✗	✓	Positive for MON87701 and MON87769
✗	✗	✓	✓	Positive for MON87708 and MON87769
✓	✓	✓	✗	Positive for DAS-44406-6, MON87701, and MON87708
✓	✓	✗	✓	Positive for DAS-44406-6, MON87701, and MON87769
✓	✗	✓	✓	Positive for DAS-44406-6, MON87708, and MON87769
✗	✓	✓	✓	Positive for MON87701, MON87708, and MON87769
✗	✗	✗	✗	Negative for DAS-44406-6, MON87701, MON87708, and MON87769

Note: A prerequisite for the unambiguous discrimination of DAS-44406-6, MON87701, MON87708, and MON87769 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 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.	<ul style="list-style-type: none"> Set Channel settings to FAM, HEX, ROX, or Cy5.
	Pipetting errors.	<ul style="list-style-type: none"> Check for correct reaction setup. Repeat the PCR run. Always run a positive control along with your samples.
	No data acquisition programmed.	<ul style="list-style-type: none"> Check the cycle programs.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> Store the foodproof GMO Soya Identification 1 lyophilized PCR Mix at 2 °C to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> Always resuspend lyophilized PCR mix thoroughly.
Negative control samples are positive.	Carry-over contamination.	<ul style="list-style-type: none"> 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. Resuspended 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 (<i>e.g.</i> , 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.	<ul style="list-style-type: none"> 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 Soya Identification 1 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 channels FAM (DAS-44406-6), HEX (MON87701), ROX (MON87708), and Cy5 (MON87769). The foodproof GMO Soya Identification 1 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 based on the requirements of ISO 21569 and the German Genetic Engineering Law § 28b GenTG for the detection of genetically modified DNA sequences [1, 2].

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 DNA fragments specific for the GM soya events DAS-44406-6, MON87701, MON87708, and MON87769.
2. The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probes due to the 5'-nuclease activity of the Taq DNA polymerase. The probes are 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 probes hybridize to an internal amplicon sequence and are cleaved by the 5'-nuclease activity of the Taq DNA polymerase. This cleavage of the probes 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 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 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 GMO Soya Identification 1 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 agricultural 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, regulatory frameworks dedicated to GMOs (e.g., Europe [3]). In order to take such frameworks into account, reliable methods for GMO testing in food products are required.

Expanding the foodproof GMO product series, in particular the GMO Screening Kits, the foodproof GMO Identification LyoKits offer the possibility to detect GM plants that do not contain one of the commonly used screening elements, e.g., events like MON87708 or BPS-CV127-9. The result is a direct identification of the targeted events. The foodproof GMO Soya Identification 1 LyoKit provides a simple and rapid molecular method



for the simultaneous detection of the event-specific sequences of four GM soya events (DAS-44406-6, MON87701, MON87708, and MON87769) in DNA preparations from raw material and food samples.

4.5 References

1. ISO 21569:2013, Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods. *International Organization of Standardization*. 2013-08.
2. BVL G 30.40-15, Screening for genetically modified soybean lines (MON87701, MON87708, MON87769, DP-305423, CV-127, DAS-68416) in plant material using multiplex real-time PCR - event-specific methods. In: *Official Compendium of Analytical Methods according to § 28b GenTG*. Berlin: Beuth Verlag GmbH. 2017-03.
3. 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.

4.6 Quality Control

The foodproof GMO Soya Identification 1 LyoKit is function tested using the LightCycler 480 System (KIT230095) and Mx3005P (Kit230096).

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.



5.4 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 602 24

6. Change Index

Version 1, November 2017:

First version of the package insert.

Revision A, December 2023:

Rebranding and new layout.

R 602 24 20 -> INS-KIT230095-96-RevA



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