foodproof® CaMV Detection Kit

Revision A, January 2023

PCR kit for the qualitative detection of cauliflower mosaic virus (CaMV) using real-time PCR instruments.

Product No. KIT230016

Kit for 96 reactions

Store the kit at -15 to -25 °C

For GMO 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.

1.2 Storage and Stability

- Store the kit at -15 to -25 °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

Vial	Label	Contents / Function / Storage
1 yellow cap	foodproof CaMV - Master Mix -	• 3 x 650 µL • Ready-to-use primer and 5'-nuclease probe mix specific for CaMV. • For amplification and detection of CaMV. • Contains Taq DNA Polymerase and Uracil-DNA N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination. • Contains a yellow dye for better visualization of the PCR mix in white PCR plates. • Store at -15 to -25 °C. • **Avoid repeated freezing and thawing!** • **Protect from light!**
2 purple cap	foodproof CaMV - Control Template -	• 1 x 50 µL • Contains a stabilized solution of DNA. • For use as a PCR positive control. • Store at -15 to -25 °C. • After first thawing store at 2 °C to 8 °C for up to one month.
3 colorless cap	foodproof CaMV - H_2O PCR-grade -	• 1 x 1 mL • Nuclease-free, PCR-grade H_2O. • For use as a PCR run negative control. • Store at -15 to -25 °C.

1.4 Product Description

The foodproof CaMV Detection Kit provides PCR primers and hydrolysis probes (5´-nuclease probes), and convenient premixed reagents for sequence specific amplification and detection of the Cauliflower mosaic virus (CaMV).
Results are obtained within 100 minutes. The foodproof CaMV Detection Kit is specifically adapted for PCR using real-time PCR instruments.

1.5 Test Principle

The cauliflower mosaic virus is identified by detecting a DNA fragment of the ORFIII with specific pairs of primers and hydrolysis probes (5´-nuclease probes) using a real-time PCR instrument.

The basic steps of the test are as follows:

Step	Description
1	Using the kit's supplied sequence-specific primers in a polymerase chain reaction (PCR), the real-time PCR instrument and its associated reagents amplify and simultaneously detect a fragment of the open reading frame III (ORFIII) of the cauliflower mosaic virus.
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 downstream from one of the primer sites and is degraded 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 real-time PCR instrument measures the emitted fluorescence of the reporter dye FAM.

1.6 Application

The foodproof CaMV Detection Kit is intended for Food and GMO testing purposes only.

1.7 Background Information

Real-time polymerase chain reaction is commonly used to screen for and quantify GM fractions in food and feed samples. The 35S promoter sequence of the cauliflower mosaic virus is one of the most used PCR targets for screening tests in GMO routine analysis. Therefore, a specific detection of the donor organism is required to avoid false positive results and further identification and quantification tests.

2. Procedure

2.1 Before You Begin

2.1.1 Precautions and Warnings

Detection of CaMV using the foodproof CaMV Detection Kit requires DNA amplification by PCR. The kit provides all required reagents in a ready-to-use master mix for the performance of PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions:

- Prepare appropriate aliquots of the kit solutions and keep them 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-filter 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: Protect the Master Mix (vial 1, yellow cap) from light.

2.1.2 Additional Equipment and Reagents Required

- Real-time PCR instruments with a FAM detection channel
- Real-time PCR compatible tubes, strips or plates with optical cap or foil applicable for the PCR-cycler in use
- Standard swing bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.
- foodproof Sample Preparation Kit III (Product No. INS230097)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Sterile reaction tubes for preparing PCR mixes and dilutions

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 detection channel. The performance of the kit was tested with the LightCycler® 96 and the PikoReal 24 real-time PCR instrument.

2.1.3 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 please refer to the corresponding product package inserts of a suitable sample preparation kit (see Additional Equipment and Reagents required).

2.1.4 Assay Time

Procedure	Time
PCR setup	15 min
PCR run	100 min (e.g., LC 480 II)
Total assay time	115 min

2.1.5 Positive Control

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

2.1.6 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 Setup

Program the PCR instrument before preparing the reaction mixes. The amplification is carried out according to the following temperature-time program (for details on how to program the experimental protocol, see the operation manual of your real-time PCR cycler):

Program for the Roche LightCycler® 480:

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

*Fluorescence detection in step 2

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

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 determined. The foodproof CaMV Detection Kit contains probes with a nonfluorescent quencher and no passive reference dye.

NOTE: 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 must be modified to 'x1'.

2.3 Preparation of the PCR Mixes

Proceed as described below to prepare a 25 µL standard reaction.
Do not touch the upper surface of the PCR plate.

1. Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening.
2. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
   - Pipet 20 µL PCR master mix into each well.
   - For the samples of interest, add up to 5 µL sample DNA (if less than 5 µL, add H_2O to 5 µL) to a well.
   - For the positive control, add 5 µL Control Template (vial 2, purple cap) to a well.
   - For the negative control, add 5 µL H_2O PCR-grade (vial 3, colorless cap).
3. Seal the plate accurately with an optical sealing foil.
4. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 seconds.
5. Cycle the samples as described above.

2.4 Analysis

The amplification of DNA of the CaMV target element is analyzed in the fluorescence channel suitable for FAM labeled probe detection.

2.5 Related Procedures

2.5.1 Prevention of Carry-over Contamination

The heat-labile Uracil-DNA 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 of 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 genomic DNA from food or plant material) 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 CaMV Detection Kit, decontamination can be achieved with the provided reagents.

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.
	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.
	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., 2.5 µL instead of 5 µL).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the Master Mix, (vial 1, yellow cap) as indicated in Kit Contents Table, protect from light. • Avoid repeated freezing and thawing.
	Master Mix is not homogeneously mixed.	• Mix the Master Mix, (vial 1, yellow cap) thoroughly before pipetting.
	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.
Fluorescence intensity varies.	Insufficient centrifugation of the plate.	• Always centrifuge the plate as described.
	Surface of the sealing foil is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the plate.

4. Supplementary Information

4.1 Ordering Information

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

4.2 License Notice

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4.3 Trademarks

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4.4 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.

4.5 Reference Number

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5. Change Index

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First version of the package insert.

Revision A, January 2023:
Rebranding and new layout.
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