



For food testing purposes
FOR *IN VITRO* USE ONLY

foodproof[®] GMO Screening Kit
(35S, NOS, bar, FMV)

- 5'Nuclease -

Version 3, March 2017

PCR kit for the qualitative detection of genetically modified plants using real-time PCR instruments.

Order No. R 302 17

Kit for 2 x 64 reactions

Store the kit at -15 to -25 °C

Table of Contents

1.	What this Product Does.....	4
	Number of Tests.....	4
	Storage and Stability.....	4
	Kit Contents.....	4
	Additional Equipment and Reagents Required.....	5
	Applicability Statement.....	5
2.	How to Use this Product.....	6
2.1	Before You Begin.....	6
	Precautions.....	6
	Sample Material.....	6
	Positive Control.....	6
	Negative Control.....	7
2.2	Procedure.....	7
	Program Setup.....	7
	Preparation of the PCR Mix.....	8
2.3	Analysis.....	9
	Color Compensation.....	9
	Data Interpretation.....	9
3.	Troubleshooting.....	11
4.	Additional Information on this Product.....	12
	How this Product Works.....	12



Table of Contents

Test Principle.....	12
Prevention of Carry-Over Contamination.....	12
Background Information.....	13
Product Specifications.....	13
References.....	13
Quality Control.....	13
5. Supplementary Information.....	14
5.1 Ordering Information.....	14
5.2 License.....	14
5.3 Trademarks.....	14
5.4 Contact and Support.....	14
6. Change Index.....	14

1. What this Product Does

Number of Tests

The kit is designed for 128 reactions (*i.e.*, 64 reactions with the 35S/NOS/bar/FMV Master Mix, [vial 1, yellow cap], and 64 reactions with the Plant Gene Master Mix, [vial 2, green cap]) with a final reaction volume of 25 µl each.

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:

Kit Contents

Vial /Cap Color	Label	Contents / Function / Storage
1 yellow cap	foodproof GMO Screening Kit - 35S/NOS/bar/FMV Master Mix -	<ul style="list-style-type: none"> • 2 x 600 µl • Ready-to-use primer and 5'Nuclease probes mix specific for the 35S-promoter of cauliflower mosaic virus (CaMV), the 3'-untranslated region of the nopaline synthase gene of <i>Agrobacterium tumefaciens</i> (NOS terminator), the <i>bar</i> resistance gene (phosphinothricin N-acetyltransferase) of the soil bacterium <i>Streptomyces hygroscopicus</i> and the FMV-promoter of the figwort mosaic virus. • For amplification and detection of the 35S, NOS, <i>bar</i> and/or FMV sequences. • Store at -15 to -25°C • Avoid repeated freezing and thawing! • Protect from light!
2 green cap	foodproof GMO Screening Kit - Plant Gene Master Mix -	<ul style="list-style-type: none"> • 2 x 600 µl • Ready-to-use primer and 5'Nuclease probes mix specific for the native plant DNA. • For amplification and detection of native plant DNA and the plant-specific Internal Control (IC). • Store at -15 to -25°C. • Avoid repeated freezing and thawing! • Protect from light!
3 red cap	foodproof GMO Screening Kit - Enzyme Solution -	<ul style="list-style-type: none"> • 4 x 32 µl • Contains Taq DNA Polymerase and Uracil-DNA Glycosylase (heat labile) for prevention of carry-over contamination. • Store at -15 to -25°C.
4 purple cap	foodproof GMO Screening Kit - Control Template -	<ul style="list-style-type: none"> • 2 x 50 µl • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at -15 to -25°C. • After first thawing store at +2 °C to +8 °C for up to one month.
5 colorless cap	foodproof GMO Screening Kit - H ₂ O, PCR-grade -	<ul style="list-style-type: none"> • 2 x 1 ml • Nuclease-free, PCR-grade H₂O for dilution of reaction mixtures. • For use as a PCR run negative control. • Store at -15 to -25°C.



Vial /Cap Color	Label	Contents / Function / Storage
6 black cap	foodproof GMO Screening Kit - Dye solution -	<ul style="list-style-type: none"> • 2 x 32µl • Contains a yellow dye for better visualization of the PCR mix in white PCR plates. • For use with the 35S/NOS/bar/FMV Master Mix
7 white cap	foodproof GMO Screening Kit - Internal Control -	<ul style="list-style-type: none"> • 2 x 32µl • Contains a stabilized solution of plasmid DNA and a yellow dye for better visualization of the PCR mix in white PCR plates. • For use as an internal amplification control with the Plant Gene Master Mix • Store at -15 to -25°C. • After first thawing store at +2 °C to +8 °C for up to one month.

Additional Equipment and Reagents Required

- real-time PCR instruments with a FAM, a VIC/HEX, a ROX and a Cy5 detection channel.
- real-time PCR compatible tubes, strips or plates with optical cap or foil applicable for the PCR-cycler in use.
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.
- Standard swing bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.

- **foodproof** GMO Sample Preparation Kit* (Order No. S 400 06)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Sterile reaction tubes for preparing PCR mixes and dilutions

Applicability Statement

The **foodproof** GMO Screening Kit is intended for the rapid detection of one or more of the four inserted primary control sequences or genes (*i.e.* 35S, NOS, *bar* gene or FMV) 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 VIC/HEX, a ROX and a Cy5 detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480 II (Roche Diagnostics) and Mx3005P® QPCR System (Stratagene).

Note:

A Color Compensation (Color Compensation Set 3*; Order No. A 500 10) is necessary and will be supplied by BIOTECON Diagnostics for users of the LC 480 Systems I and II. Please contact BIOTECON Diagnostics for further information.

* Available from BIOTECON Diagnostics; see Ordering Information



2. How to Use this Product

2.1 Before You Begin

Precautions

Detection of genetically modified DNA using the **foodproof** GMO Screening Kit requires DNA amplification by PCR. The kit provides all the 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:

- 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-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 35S/NOS/bar/FMV Master Mix, (vial 1, yellow cap) and the Plant Gene Master Mix, (vial 2, green cap) away from light.

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).

Positive Control

Always run a positive control (one for the 35S/NOS/bar/FMV PCR and one for the plant gene PCR) with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [Control Template, (vial 4, purple cap)].



Negative Control

Always run negative controls (one for the 35S/NOS/bar/FMV PCR and one for the plant gene PCR) with the samples. To prepare negative controls, replace the template DNA with H₂O, PCR-grade (vial 5, colorless cap). Furthermore, it is recommended to 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.

Note: Due to the sensitivity of the Plant Gene Master Mix, and because most reagents used for sample preparation contain minute amounts of plant DNA, false-positive results in sample preparation negative controls cannot be excluded. If so, the actual analytical samples should have a lower crossing point than the sample preparation control.

2.2 Procedure

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:

<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

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** GMO Screening Kit contains probes with a non fluorescent ("dark") 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 has to be modified to "x1".

Note: The kit can also be used with the protocol of the **foodproof** GMO Soya Quantification Kit , 5'Nuclease (R 302 19).



Preparation of the PCR Mixes

Proceed as described below to prepare a 25 µl standard reaction. The PCR mixes for the 35S/NOS/bar/FMV-PCR and the Plant-PCR must be set-up separately, using the respective Master Mixes. 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. Mix carefully but thoroughly by pipetting up and down.
2. In a 1.5 ml reaction tube, prepare the PCR Mix by adding the following components in the order mentioned below, then mix gently but thoroughly by pipetting up and down:

The volumes indicated below are based on a single 25 µl standard reaction. Prepare the PCR mix by multiplying the amount in the "Volume" column by the number of reactions (sample and control reactions) to be cycled plus one or two additional reactions to cover pipetting losses.

Mix for the 35S/NOS/bar/FMV - PCR:

Component	Volume
35S/NOS/bar/FMV Master Mix, (vial 1, yellow cap)	18 µl
Enzyme Solution, (vial 3, red cap)	1 µl
Dye solution, (vial 6, black cap)	1 µl
Total volume	20 µl

Mix for Plant Gene - PCR:

Component	Volume
Plant Gene Master Mix, (vial 2, green cap)	18 µl
Enzyme Solution, (vial 3, red cap)	1 µl
Internal Control, (vial 7, white cap)	1 µl
Total volume	20 µl

3. Mix carefully but **thoroughly** by pipetting up and down. Do not vortex.
 - Pipet 20 µl PCR mix into each well.
 - For the samples of interest, add up to 5 µl sample DNA (if less than 5 µl add H₂O to 5 µl) to a well.
 - For the negative control, add 5 µl H₂O, PCR-grade (vial 5, colorless cap).
 - For the positive control, add 5 µl **foodproof** GMO Screening Control Template (vial 4, purple cap).
4. Seal the Plate accurately with an optical sealing foil.
5. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s.
6. Cycle the samples as described above.

2.3 Analysis

Color Compensation

The use of the previously generated color compensation file or color compensation object is a prerequisite for the unambiguous discrimination of 35S, NOS, *bar* and FMV with the 35S/NOS/*bar*/FMV-PCR and of plant DNA and internal control amplification with the Plant Gene-PCR in this multi-color experiment with the LightCycler 480 instrument. For information on the generation and use of a color compensation file or object, refer to the LightCycler® 480 System Operator's Manual.

Data Interpretation

The amplification of DNA of the four GMO target elements is analyzed in the fluorescence channels suitable for FAM (35S promoter), VIC/HEX (NOS terminator), ROX (*bar* gene) and Cy5 (FMV promoter) labeled probes detection. In addition the amplification of plant DNA and of the Internal Control is analyzed in the fluorescence channels suitable for FAM (plant DNA) and VIC/HEX (Internal Control).

foodproof Plant Gene Master Mix		
Result in Channel FAM Plant gene	Result in Channel VIC/HEX Internal Control	Result interpretation
Positive	Positive or Negative	Positive for plant DNA
Negative	Positive	Negative for plant DNA
Negative	Negative	Invalid

foodproof 35S/NOS/bar/FMV Master Mix					
Result in Channel					
FAM 35S promoter	VIC/HEX NOS terminator	ROX bar gene	Cy5 FMV promoter	VIC/HEX Internal Control - Plant Gene Master Mix -	Result Interpretation
Positive	Negative	Negative	Negative	Positive/Negative	Positive for 35S
Negative	Positive	Negative	Negative	Positive/Negative	Positive for NOS
Negative	Negative	Positive	Negative	Positive/Negative	Positive for <i>bar</i>
Negative	Negative	Negative	Positive	Positive/Negative	Positive for FMV
Negative	Negative	Negative	Negative	Negative	Invalid
Negative	Negative	Negative	Negative	Positive*	Negative for GMO

*The result for the Internal Control can also be negative in case of a positive signal for amplification of plant DNA.

Note:

Use the "High Sensitivity" setting of the LightCycler Software to calculate results. Nevertheless, always check the software results (red signals for positive samples/green signals for negative samples) for plausibility by inspection of the amplification curves.

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, VIC/HEX, ROX and Cy5
	Pipetting errors or omitted reagents.	<ul style="list-style-type: none"> 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.	<ul style="list-style-type: none"> 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).	<ul style="list-style-type: none"> Use the recommended DNA sample preparation kit to purify template DNA. Dilute samples. Always run a positive control along with your samples.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> Store the 35S/NOS/bar/FMV Master Mix, (vial 1, yellow cap) and the Plant Gene Master Mix, (vial 2, green cap) as indicated in Kit Contents Table, protect from light. Avoid repeated freezing and thawing.
	35S/NOS/bar/FMV Master Mix or Plant Gene Master Mix are not homogeneously mixed.	Mix the 35S/NOS/bar/FMV Master Mix, (vial 1, yellow cap) and Plant Gene Master Mix, (vial 2, green 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.	<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.
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. Additional Information on this Product

How this Product Works

The **foodproof** GMO Screening Kit provides primers and 5'Nuclease probes (for sequence-specific detection), convenient premixed reagents, and a control template for reliable interpretations of results. The **foodproof** GMO Screening Kit minimizes contamination risk and contains all reagents (except for template DNA) needed for screening for genetically modified plants. To ensure maximum reliability of the detection system and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is supplied (vial 7, white cap). The IC has to be added to each reaction of the Plant-PCR. A 5'Nuclease Probe was designed to bind specifically to the IC, allowing detection in the VIC/HEX channel, whereas the plant DNA is detected in the FAM channel. In case of a negative result due to inhibition of 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 DNA of genetically modified plants in the sample. Primers and 5'Nuclease probes provide specific detection of the 35S, NOS, *bar* and FMV sequence in food, feed and seed samples. The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM, VIC/HEX, ROX and Cy5 detection channel.

Test Principle

1. Using the kit's supplied sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and its associated reagents amplify and simultaneously detect fragments of the 35S, NOS, *bar* and/or FMV sequence in one well of a microtiter plate and a fragment of a native plant gene in a second well.
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 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 probe hybridizes to an internal sequence of the amplicon downstream from one of the primer sites 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 real-time PCR instrument measures the emitted fluorescence of the reporter dye.

Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA 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 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 Screening Kit, decontamination can be achieved with the provided reagents.



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 (*e.g.*, Europe [1]) established, or are currently in the process of establishing, regulatory frameworks dedicated to GMOs. In order to take such frameworks into account, reliable methods for GMO screening in food products are required. The **foodproof** GMO Screening Kit provides a simple and rapid molecular method for the simultaneous detection of the 35S, NOS, *bar* and FMV sequences in DNA preparations from raw material and food samples. The 35S-promoter of the cauliflower mosaic virus (CaMV), the 3'-untranslated region (terminator) of the nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*, the phosphinothricin N-acetyltransferase (PAT) encoding *bar* gene from *Streptomyces hygroscopicus* and the 35S-promoter of the figwort mosaic virus (FMV) are the four most commonly used sequences of genetically modified plants. Most of the presently available GMO crops are positive for either the 35S-promoter, the NOS-terminator, the *bar* gene or the FMV-promoter or several of them together.

Product Specifications

The 35S/NOS/*bar*/FMV Master Mix, (vial 1, yellow cap) is sequence-specific for the 35S-promoter of cauliflower mosaic virus, the 3'-untranslated region of the nopaline synthase gene from *Agrobacterium tumefaciens* (NOS-terminator) the phosphinothricin N-acetyltransferase (PAT) encoding *bar* gene from *Streptomyces hygroscopicus* and the 35S-promoter of the figwort mosaic virus (FMV). The Plant Gene Master Mix, (vial 2, green cap) is sequence specific for a highly conserved plant gene.

Note: *Agrobacterium tumefaciens* and the cauliflower mosaic virus are plant pests reported to infest non-transgenic plants (2). Therefore, false-positive results cannot be excluded.

References

1. Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal L 043, 14/02/1997 p. 0001–0007.
2. Lipp M, Brodmann P, Pietsch K, Pauwels J and Anklam E. 1999. IUPAC Collaborative trial study of a method to detect the presence of genetically modified soybeans and maize dried powder. Journal of AOAC International 82, 923–928.

Quality Control

The **foodproof** GMO Screening Kit is function tested using the LightCycler® 480 Instrument II.



5. Supplementary Information

5.1 Ordering Information

BIOTECON Diagnostics is offering a broad range of reagents and services. For a complete overview and for more information, please visit our website at www.bc-diagnostics.com.

5.2 License

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.

5.3 Trademarks

foodproof® is a trademark of BIOTECON 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 BIOTECON Diagnostics, please contact our Technical Support staff (for details see www.bc-diagnostics.com). 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. Change Index

Version 1

First version of the package insert.

Version 2, July 2010

Page 7: Second PCR program for cyclers other than the LightCycler® 480 added. NOTE for users of the Agilent Mx3005P instrument added.

Version 3, March 2017

License Notice changed.

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