foodproof® GMO A2704-12 Soya Quantification Kit

Revision A, May 2025

PCR kit for the quantitative detection of genetically modified soya event A2704-12 (OECD unique identifier ACS-GMØØ5-3) using real-time PCR instruments.

Product No. KIT230019

Kit for 120 reactions for a maximum of 48-60 samples

Store the kit at -15 to -25 °C

For GMO testing purposes
FOR IN VITRO USE ONLY

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1. Product Overview

1.1 Number of Tests

The kit is designed for 128 reactions [i.e., 64 reactions with the GMO Gene Master Mix, (vial 1, yellow cap) and 64 reactions with the Reference Gene Master Mix, (vial 2, green cap)] with a final reaction volume of 25 µL each.

Note: The maximum number of samples that can be analyzed per experiment depends on the chosen quantification procedure:

Procedure A Including relative standard curves	Procedure B Importing external relative standard curves
Quantification of up to 48 samples (single sample preparation) in two runs plus calibration curves, one negative control reaction, and one calibrator per run.	Quantification of up to 60 samples (single sample preparation) in two runs plus calibrator and one negative control reaction per run.

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® GMO A2704-12 Soya - GMO Gene Master Mix -	• 2 x 650 µL • Ready-to-use primer and 5'-nuclease probe mix specific for the integration region of the construct inserted into the soya genome • For amplification and detection of the A2704-12 soya event • 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 green cap	foodproof GMO A2704-12 Soya - Reference Gene Master Mix -	• 2 x 650 µL • Ready-to-use primer and 5'-nuclease probe mix specific for the lectin gene (*Le1*) of native soya • For amplification and detection of the *Le1* gene • 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!**

Vial	Label	Contents / Function / Storage
3 purple cap	foodproof GMO A2704-12 Soya - Calibrator DNA -	• 2 x 50 µL • Contains a stabilized solution of plasmid DNA • For use as a PCR run calibrator and positive control • Store at −15 to −25 °C • After first thawing, store at 2 to 8 °C for up to one month
4 blue cap	foodproof GMO A2704-12 Soya - Dilution Buffer -	• 4 × 1 mL • For dilution of calibrator and sample DNA • Store at −15 to −25 °C
5 colorless cap	foodproof GMO A2704-12 Soya - H2O PCR-grade -	• 2 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® GMO A2704-12 Soya Quantification Kit provides PCR primers and hydrolysis probes (5'-nuclease probes), and convenient premixed reagents for sequence-specific amplification and detection of the integration region of the construct inserted into the plant genome (GM-specific DNA) and the soybean endogenous *Le1* gene (taxon-specific DNA). A Calibrator DNA is also provided with the kit. It serves as a positive control and as a reference to normalize the relative DNA in the sample. This is necessary to quantify the percentage ratio of the copy number of gene-specific DNA to the number of taxon-specific DNA in a single run (i.e., to ensure accurate determination of the DNA copy number ratio of the GM-specific DNA to the taxon-specific DNA, expressed in %). Optimized PCR conditions allow analysis of the GM-specific and the taxon-specific PCR in a single run. Results are obtained within 100 minutes.

Normalization corrects for differences in GMO content values, resulting from the combined variation in the quantity and quality of DNA samples and the efficiency of the PCR. The foodproof GMO A2704-12 Soya Quantification Kit is specifically adapted for PCR using real-time PCR instruments.

- The GMO Gene Master Mix, (vial 1, yellow cap) allows the amplification and detection of a fragment of the integration region of the construct of the genetically modified A2704-12 soya using specific primers.
- A fragment of the native soya lectin gene (*Le1*) is amplified and detected with the Reference Gene Master Mix (vial 2, green cap). The reaction product serves both as a control for DNA integrity and as a reference for relative quantification.

1.5 Test Principle

A2704-12 soya (LibertyLink, OECD unique identifier ACS-GMØØ5-3) is identified by detecting a DNA fragment of the unique integration region of the construct inserted into the soya genome. To detect native soybean, a soya-specific lectin gene (*Le1*) is amplified and detected with the Reference Gene Master Mix, which serves as both a control for DNA integrity and as a reference for relative quantification. Both amplicons are detected in separate reactions 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 fragments of genetically modified A2704-12 soya and native soya, respectively.
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.
5	Determination of the relative DNA copy number ratio of the genetically modified A2704-12 soya event. This can be performed using one of the following alternative procedures: • Procedure A: Including relative standard curves into the run. • Procedure B: Importing external relative standard curves from a previous run.

1.6 Application

The foodproof GMO A2704-12 Soya Quantification Kit is intended for GMO testing purposes only.

The foodproof GMO A2704-12 Soya Quantification Kit is used to determine the relative DNA copy number ratio of genetically modified A2704-12 soya in flour or food. Relative quantification is performed by comparing the amplification of a specific gene of genetically modified A2704-12 soya with the amplification of a specific gene of native soya. For calculation purposes, included or external (imported) standard curves can be used.

Note: The foodproof GMO A2704-12 Soya Quantification Kit has been validated to quantify a DNA copy number ratio from 0.05% to 100% GMO content. The relative amount of 0.05% genetically modified A2704-12 soya can be detected in raw material.

1.7 Product Characteristics

Specificity	The primers and hydrolysis probes (5'-nuclease probes) provided in the GMO Gene Master Mix (vial 1, yellow cap) and in the Reference Gene Master Mix (vial 2, green cap) are sequence-specific for A2704-12 soya and native soya, respectively.
Sensitivity	Detects the relative amount of 0.05% genetically modified A2704-12 soya content in raw material.
Measuring Range	The kit can measure the relative content of A2704-12 soya event in a range of 0.05% to 100%.

1.8 Background Information

Real-time polymerase chain reaction is commonly used to quantify GM fractions in food and feed samples. This DNA-based quantification technique measures the ratio between transgenic deoxyribonucleic acid (DNA), i.e., derived from the genetic modification, and endogenous DNA, which is specific for the biological species.

The genetically engineered soybean A2704-12 contains a glufosinate tolerance through the phosphinothricin acetyltransferase gene (PAT) derived from *Streptomyces viridochromogenes*.

According to Swiss and EU law [1-2], foods and additives with more than 1% (Switzerland) and 0.9% (in other European countries) relative amount of genetically modified organisms must be labeled as GMO products.

2. Procedure

2.1 Before You Begin

2.1.1 Precautions and Warnings

Quantification of the relative GMO content using the foodproof GMO A2704-12 Soya Quantification 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-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

Note: Protect the GMO Gene Master Mix (vial 1, yellow cap) and the Reference Gene Master Mix (vial 2, green 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, available from Hygiena Diagnostics, Product No. KIT230174; see Ordering Information for more info
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with nuclease-free, aerosol-barrier pipette tips.
- 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® 480, LightCycler® 96 (Roche Diagnostics), Mx3005P®, AriaMx (Agilent Technologies), ABI 7500 Fast, QuantStudio™ 5 (Thermo Fisher Scientific), and Dualo 32® R2 (Hygiena®).

2.1.3 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For the 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).

Note: In order to quantify the relative amount of genetically modified soya in a sample of interest, **the sample DNA must be diluted at least 1:4 in the Dilution Buffer** (vial 4, blue cap) provided with the kit. This dilution step is essential to compensate for the different ion concentrations of the Calibrator DNA and the sample DNA. The latter depends on the buffers used for the sample preparation procedure. This dilution step also reduces the risk of inhibitory effects on the PCR reaction.

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 Calibrator DNA (vial 3, purple cap) or with a positive sample preparation control (e.g., Certified Reference Material).

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 5, 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 operation manual):

Program:

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

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 A2704-12 Soya Quantification 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 has to be modified to 'x1'.

2.3 Experimental Setup

2.3.1 General Remarks

Determination of the relative ratio content of A2704-12 soya can be performed using one of the following alternative procedures:

- Procedure A: Quantification using included (in-run) relative standard curves
- Procedure B: Quantification using external (imported) relative standard curves

Thoroughly read the software instructions prior to performing this assay.

2.3.2 Procedure A – Quantification Using Included (In-run) Relative Standard Curves

Each individual real-time PCR run consists of:

- Six dilution steps of the Calibrator DNA for both the GMO gene and the reference gene PCR in order to generate the respective calibrator curves (see table below).
- A variable number of sample preparations to be analyzed for genetically modified soya DNA amplification.
- At least one negative control reaction to control for contamination of the GMO Gene and the Reference Gene Master Mix, respectively.
- One positive reaction with the Calibrator DNA each for the GMO gene and the reference gene PCR to compensate for constant differences between the PCR performance of the GMO gene and the reference gene.

Therefore, a typical experiment consists of 16 wells needed for controls, plus 2 × (n) wells needed for the samples of interest, where (n) indicates the number of food samples of interest. Since a multi-well plate has 96 wells, 40 food samples can be analyzed during one PCR run if the GMO gene and the reference gene are analyzed in the same run.

2.3.3 Dilution of Calibrator DNA

Quantification of the GMO content via Procedure A requires the stepwise dilution of the Calibrator DNA (vial 3, purple cap) in the Dilution Buffer (vial 4, blue cap) as shown below:

Dilution Step	Dilution	Concentrations to be Entered as Standards for the Reference Gene or the GMO Gene PCR
1	Undiluted	100
2	1:4	25
3	1:16	6.25
4	1:64	1.56
5	1:256	0.39
6	1:1024	0.098

2.3.4 Procedure B – Quantification Using External (Imported) Relative Standard Curves

Each individual real-time PCR run consists of:

- One positive reaction with the Calibrator DNA each for the GMO gene and the reference gene PCR to compensate for constant differences between the PCR performance of the GMO gene and the reference gene.
- A variable number of sample preparations to be analyzed for genetically modified soya DNA amplification.
- At least one negative control reaction to control for contamination of the GMO Gene and the Reference Gene Master Mix, respectively.

Therefore, a typical experiment consists of 4 wells needed for controls, plus 2 x (n) wells needed for the samples of interest, where (n) indicates the number of food samples of interest. Since a multi-well plate has 96 wells, 46 food samples can be analyzed during one PCR run if the GMO gene and the reference gene are analyzed in the same run.

Note: Procedure B is only applicable if the real-time PCR instrument allows importing external standard curves generated in a previous run.

2.4 Preparation of the PCR Mixes

Proceed as described below to prepare a 25 µL standard reaction. The PCR assays for the GMO gene and the Reference gene 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.
2. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
   - Pipette 20 µL PCR master mix into each well.
   - For the samples of interest, add up to 5 µL sample DNA to a well (if less than 5 µL add H_2O to 5 µL).
   - For the negative control, add 5 µL H_2O, PCR-grade (vial 5, colorless cap).
   - *Procedure A*: For the included (in-run) relative standard curves, add 5 µL of each dilution of Calibrator DNA (vial 3, purple cap) to the wells.
   - *Procedure B*: For the positive control, add 5 µL Calibrator DNA (vial 3, purple cap) to a well.
3. Seal the plate accurately with an optical sealing foil or with the lid strips, depending on which plastic is used.
4. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s.
5. Cycle the samples as described above.

2.5 Calculation of Relative Amount of Genetically Modified Soya

2.5.1 Procedure A – Quantification Using Included (In-run) Relative Standard Curves

The use of calibration curves results in two values for every sample analyzed (i.e., one for the GMO gene and one for the reference gene).

Note: Since the calibration curves are specific for the GMO and reference PCR, respectively, it is important that the generated values for the GMO and reference PCR are distinguishable. The percentage of A2704-12 soya relative to the total soya content within the sample of interest must be calculated manually, with a spreadsheet program or with the analysis software of the real-time PCR instrument used (e.g., LightCycler 480 instrument, Mx3005P system). Please refer to the manual of the real-time PCR instrument for more detailed

information. The calculation of the relative GMO content is based upon the resulting crossing points or Ct-values of one particular sample and the efficiency of the PCR. In brief, the crossing point or Ct-value is the cycle at which PCR amplification begins its exponential phase and is considered the point that is most reliably proportional to the logarithm of the initial concentration. The efficiency of the PCR describes the kinetics during the reaction. The overall reaction efficiency is represented by the slope of the calibration curve. Since primers and hydrolysis probes (5'-nuclease probes) for both parameter-specific components (GMO and reference gene) have individual PCR efficiencies, a calibration curve for each gene must be generated.

Notes:

- Quantify two independent sample preparations of each food sample and take the mean value as the final result.
- The ratio of GMO to reference DNA in the Calibrator DNA provided with the kit is 1.0.
- Due to statistical reasons, the A2704-12 soya detection and quantification becomes less reliable at low copy numbers, (i.e., results obtained from sample material with crossing points or Ct-values greater than 38 for either the GMO and/or the reference gene). Crossing points or Ct-values greater than 29 in the reference PCR indicate there is not enough soya DNA in the sample to reliably quantify 1% GMO content.
- DNA degradation during food processing may affect GMO quantification.

2.5.2 Procedure B – Quantification Using External (Imported) Relative Standard Curves

For some real-time PCR instruments, it is possible to import external relative standard curves from a previously generated PCR run with the same instrument (e.g., LightCycler 480 instrument).

2.6 Related Procedures

2.6.1 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 A2704-12 Soya Quantification 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 pipette 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 GMO Gene Master Mix (vial 1, yellow cap) and the Reference Gene Master Mix (vial 2, green cap) as indicated in Kit Contents Table; protect from light. • Avoid repeated freezing and thawing.
	GMO Gene Master Mix or Reference Gene Master Mix is not homogeneously mixed.	• Mix the GMO Gene Master Mix (vial 1, yellow cap) and the Reference 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.	• 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.

3.2 References

1. REGULATION (EC) No 1829/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 22 SEPTEMBER 2003, on genetically modified food and feed.
2. REGULATION (EC) No 1830/2003 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL OF 22 SEPTEMBER 2003, concerning the traceability and labeling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC.7.

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

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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

The reference number and original Hygiena Diagnostics GmbH article number: F 302 66

5. Change Index

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First version of the product instructions with Hygiena branding and new layout. F 302 66 -> INS-KIT230019-REVA

Hygiena®
Camarillo, CA
93012 USA

www.hygiena.com/support

Manufactured by
Hygiena Diagnostics GmbH

Hermannswerder 17

14473 Potsdam
Germany
www.hygiena.com