foodproof® Gluten Detection Kit

Revision A, December 2023

PCR kit for the qualitative detection of cereal DNA containing gluten using real-time PCR instruments.

Product No. KIT230061

Kit for 64 reactions for a maximum of 62 samples

Store the kit at -15 to -25 °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 64 reactions [Master Mix (vial 1, yellow cap)] with a final reaction volume of 25 µL each. Up to 62 samples plus one positive control [Control Template (vial 2, purple cap)] and one negative control [PCR-grade water (vial 3, colorless cap)] can be analyzed.

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® Gluten Detection Kit - Master Mix -	• 2 x 650 µL • Ready-to-use primer and 5'-nuclease probe mix for the amplification of gluten-containing cereal specific DNA and the internal control (plasmid DNA) • Contains Taq DNA-Polymerase and Uracil-DNA N-Glycosylase (UNG; prevention of carry-over contamination) • Yellow dye improves the visualization of the Master Mix in PCR tubes and plates • Store at -15 to -25 °C • **Avoid repeated freezing and thawing!** **Protect from light!**
2 purple cap	foodproof Gluten Detection Kit - Control Template -	• 2 x 50 µL • Contains a stabilized solution of plasmid DNA • For use as a PCR positive control • Store at -15 to -25 °C • After first thawing, store at 2 to 8 °C for up to one month
3 colorless cap	foodproof Gluten Detection Kit - H_2O PCR-grade -	• 1 x 1 mL • Nuclease-free, PCR-grade H_2O • For use as a PCR run negative control • After first thawing, store at 2 to 8 °C for up to one month

1.4 Product Description

The foodproof Gluten Detection Kit provides PCR primers, hydrolysis probes (5' nuclease probes), and convenient premixed reagents for the species-specific amplification and detection of DNA of gluten-containing cereals (wheat, spelt, Khorasan wheat/Kamut, rye, barley, and triticale measured in FAM). Since only traces of gluten and the linked cereals in food can trigger severe immune responses in consumers, multi-copy targets of these cereal genomes were used to increase the sensitivity of the PCR assay. Additionally, the Control Template and PCR-grade H_2O monitor the PCR run for validity. An internal control (measured in HEX) is used to evaluate possible PCR inhibitions by matrix effects.

In combination with the foodproof Sample Preparation Kit III (Product No.: KIT230174) and the foodproof Magnetic Preparation Kit III (Product No.: KIT230182), wheat, spelt, Khorasan wheat (Kamut), rye, barley, and triticale DNA can be reliably detected in difficult matrices like minced meat, spices, ice cream, and chocolate. PCR results are obtained within 100 minutes. Optimized PCR conditions allow the analysis of the specific PCR of gluten-containing cereals in a single run. The foodproof Gluten Detection Kit is specifically adapted for PCR using real-time PCR instruments.

Note: The kit described in this instruction manual has been developed for real-time PCR instruments.

1.5 Application

The foodproof Gluten Detection Kit is intended for food testing purposes only. It is used to identify low amounts of gluten-containing cereal DNA in flour or processed food and is also conceived for absolute quantifications by using the Allergen RM 800 reference material.

Note: For quantification purposes, please refer to our reference material Allergen RM 800 (Product No.: KIT230009)

1.6 Product Characteristics

Specificity	The primers and hydrolysis probes (5'-nuclease probes) provided in the Master Mix (vial 1, yellow cap) are sequence-specific for wheat, spelt, Khorasan wheat (Kamut®), rye, barley, triticale, and the Internal Control, respectively. The specificity of the assay was proven by 104 plant and animal species, as well as 27 commercial food products.
Sensitivity	The limit of detection was determined at 0.1 genome-equivalents and 0.1 - 1 ppm in spiked rice flour matrix for wheat, rye, and barley, respectively. The limit of quantification was set at 0.8 ppm.
Precision	The Repeatability Relative Standard Deviation (RSDr) of wheat in food samples measured below 33.8% for Allergen RM 800 at 800 ppm, 101.7 % (0.38ppm ±0.39) for 1 ppm in ice cream, and 79.58 % (0.93ppm ±0.74) for 1 ppm in chocolate, 76.25 % (0.93ppm ±0.71) for 1 ppm in spices and 23.73 % (1.83ppm ±0.43) for 1 ppm in minced meat. A high concentration of polyphenols is known to be present in cereals and the tested matrices (except for minced meat), leading to higher variations of results, generally.
Robustness	Reproducibility of Cp/Ct-values was analyzed with different real-time PCR instruments, including Roche LightCycler® 480 II, Agilent AriaMx, Applied Biosystems® 7500 FAST, Thermo Scientific PikoReal, and Bio-Rad iQ™ 5 Cycler (in total= 0.59 %).

Note: *More detailed information is listed in the Validation Data Report of the* ***food****proof Gluten Detection Kit. Please contact our Technical Support Team with questions.* [*Hygiena.com/support*](Hygiena.com/support)

1.7 Background Information

People affected by foodborne allergens develop abnormal immunological reactions to specific food components. These can range from mild allergic symptoms to life-threatening anaphylactic shock. Affected patients rely on avoiding the allergenic food or ingredient based on appropriately labeled food products. EU Commission Directive 2007/68/EC and 1169/2011 define 20 allergenic substances which have to be declared if contained in food products and loose goods, including gluten-containing cereals like wheat, spelt, Khorasan wheat (Kamut), rye, barley, and crossbreds.

In contrast to “gluten-free” labeling of Codex Alimentarius, EU regulation clearly requires labeling of specified gluten-containing cereals. This difference is based on the diseases that are the foundation for the regulations. While the EU directive refers to allergy, Codex Alimentarius aims for coeliac disease-affected persons. Using the Codex Alimentarius for gluten labeling, a threshold of <20 ppm exists for gluten-free products. This quantification can be realized by using the Allergen RM 800 reference material (Product No.: KIT230009) of Hygiena Diagnostics GmbH. The PCR method for gluten analysis is in agreement with the Codex Alimentarius and the respective EU regulations (DIN EN 15634-1:2009).

2. Procedure

2.1 Before You Begin

2.1.1 Precautions and Warnings

Detection of cereal DNA that contains gluten using the foodproof Gluten 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-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 Master Mix (vial 1, yellow cap) from light and avoid multiple freezing and thawing cycles.

2.1.2 Additional Equipment and Reagents Required

- Allergen RM 800 (Product No. KIT230009) reference material for quantitative purposes
- foodproof Sample Preparation Kit III (Product No. KIT230174) or the foodproof Magnetic Preparation Kit III (Product No. KIT230182)
- Real-time PCR instruments with a FAM and HEX/VIC detection channel
- Real-time PCR compatible tubes, strips or plates with optical cap or foil applicable for the PCR cycler used
- Standard swing bucket centrifuge containing a rotor for multiwell plates with suitable adaptors
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Sterile reaction tubes for preparing PCR mixes and dilutions

2.1.3 Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. The foodproof Gluten Detection Kit was validated with food products such as soup, sauces, spices, fast food, confectionary, and others (e.g., pasta, muesli, bread).

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).

2.1.4 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 (e.g., Reference Material Allergen RM 800).

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 H_2O (vial 3, colorless cap). 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.

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 specific for your real-time PCR cycler):

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

Note: For some real-time PCR instruments (e.g., ABI 7500), the type of the probe quencher as well as the usage of a passive reference dye has to be determined. The foodproof Gluten Detection Kit contains probes with a nonfluorescent 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 must be modified to 'x1'.

2.3 Preparation of the PCR Mix

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

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.
   a. Pipet 20 µL PCR Master Mix into each well
   b. For the samples of interest, add 5 µL sample DNA (if less than 5 µL add H_2O to 5 µL) to a well
   c. For the negative control, add 5 µL H_2O (PCR-grade, vial 3, colorless cap) to a well
   d. For the positive control, add 5 µL Control Template DNA (vial 2, purple cap) to a well
3. Seal the plate/strips accurately with an optical sealing foil.
4. Place the plate/strips in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s
5. Cycle the samples as described above.

2.4 Data Interpretation

The amplification of DNA of gluten-containing cereals was analyzed in the fluorescence channel FAM and the internal control in channel HEX/ VIC.

Result in Channel FAM Gluten-containing Cereals	Result in Channel HEX/ VIC Internal Control	Result Interpretation
Positive	Positive/ Negative	Positive for gluten-containing cereals
Negative	Positive	Negative for gluten-containing cereals
Negative	Negative	Invalid

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 Gluten 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 channels have been chosen.	• Set Channel settings to FAM and HEX/VIC.
	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 volume 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/strips.	• Always centrifuge the plate/strips as described.
	Surface of the sealing foil is dirty (e.g., by direct skin contact).	• Always wear gloves when handling the plate/strips.
Precipitation of the Master Mix	Incomplete thawing of the Master Mix	• Warm up the Master Mix carefully in your hands and snap gently to the tube until the precipitation is gone (do **not** vortex!).
	Precipitation of stabilizing reagents in the Master Mix	

3.2 References

1. DIN EN 15634-1:2009; Detection of food allergens by molecular biological methods.
2. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers.
3. Commission directive 2007/68/EC of 27 November 2007 amending Annex IIIa to Directive 2000/13/EC of the European Parliament and of the Council as regards certain food ingredients.
4. DIRECTIVE 2003/89/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs.
5. Codex Alimentarius: Codex standard for foods for special dietary use for persons intolerant to gluten; codex stan 118 – 1979.

4. Supplementary Information

4.1 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.

- foodproof Sample Preparation Kit III (Product No. KIT230174)
- foodproof Magnetic Preparation Kit III (Product No. KIT230182)
- Allergen RM 800 (Product No. KIT230009)

4.2 License

License Notice

NOTICE TO PURCHASER: LIMITED LICENSE

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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

The reference number and original Hygiena Diagnostics GmbH article number: R 302 64

5. Change Index

Version 1, October 2015
First version of the package insert.

Revision A, December 2023
Rebranding and new layout.
R 302 64 20 -> INS-KIT230061-RevA

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