



foodproof® Hazelnut Detection Kit

Revision A, December 2023

PCR kit for the qualitative detection of hazelnut DNA using real-time PCR instruments.

Product No. KIT230059

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



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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 [H₂O PCR-grade (vial 3, colorless cap)] can be analyzed.

1.2 Storage and Stability

- Store the kit at -15 °C 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® Hazelnut Detection Kit - Master Mix -	• 2 x 650 µL • Ready-to-use primer and 5' nuclease probe mix for the amplification of hazelnut-specific DNA and the internal control (plasmid DNA) • Contains Taq DNA Polymerase and Uracil-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 Hazelnut 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 °C to +8 °C for up to one month
3 colorless cap	foodproof Hazelnut Detection Kit - H ₂ O PCR-grade -	• 1 x 1 mL • Nuclease-free, PCR-grade H₂O • For use as a PCR run negative control • After first thawing, store at +2 °C to +8 °C for up to one month

1.4 Product Description

The foodproof Hazelnut Detection Kit provides PCR primers, hydrolysis probes (5' nuclease probes), and convenient premixed reagents for the species-specific amplification and detection of hazelnut DNA (*Corylus avellana*). As even trace amounts of hazelnut in food can trigger severe immune responses to consumers, multi-copy targets of the hazelnut genome were used to increase the sensitivity of the PCR assay. Additionally, the Control Template and PCR-grade water monitor the PCR run for validity.

In combination with the foodproof Sample Preparation Kit III (Product No. KIT230174) and the foodproof Magnetic Preparation Kit III (Product No. KIT230182), hazelnut DNA can be reliably detected in difficult matrices, such as ice cream and chocolate.

PCR results are obtained within 100 minutes.

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



1.5 Application

The foodproof Hazelnut Detection Kit is intended for food testing purposes only. Users may identify low amounts of hazelnut DNA in flour and other processed foods. Absolute quantification is possible with this kit when used in combination with Allergen RM 800 reference material.

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

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 hazelnut and the Internal Control, respectively. Specificity of the assay was proven by 94 plant and animal species, as well as 42 commercial food products.
Sensitivity	The limit of detection was determined to be 1 hazelnut genome equivalent and 1 ppm in hazelnut spiked rice flour matrix. The limit of quantification was determined to be 0.8 ppm based on the threshold set by the standard curve.
Precision	The Repeatability Relative Standard Deviation (RSDr) of high and low concentrations of hazelnut in food samples was measured to be below 13 % for Allergen RM 800 at 800 ppm, 28 % (0.6 ppm $\pm$ 0.2) for 1 ppm in ice cream, and 74 % (0.7 ppm $\pm$ 0.6) in chocolate.
Robustness	Reproducibility of Cp-values was successfully tested with different real-time PCR instruments, including Roche LightCycler® 480 II, Agilent Mx3005p, Applied Biosystems® 7500 FAST, Thermo Scientific PikoReal, and Bio-Rad iQ™5 Cyclor.

Note: More detailed information is listed in the Validation Data Report of the foodproof Hazelnut Detection Kit. Please contact our Technical Support team for information (www.hygiena.com/contact).

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 must rely on appropriately labeled food products in order to avoid the allergenic food or ingredient. EU Commission Directive 2007/68/EC defines 20 allergenic substances that must be declared if contained in food products, including hazelnut. This regulation will be extended to unpacked food (EU regulation 1169/2011). Since traces of allergenic components can cause dangerous reactions in sensitive individuals, accurate detection of allergenic food components at different stages of production and critical points in the production chain is essential.

Since no official threshold levels for food allergens exist, only qualitative analyses are mandatory. However, quantitative analyses may be used to monitor production and cleaning processes or to establish and comply with in-house threshold levels. Therefore, the foodproof Hazelnut Detection Kit was designed for both qualitative and quantitative analyses when used in combination with the Allergen RM 800 reference material (KIT230009). The detection of species that contain allergenic ingredients by molecular methods is regulated by DIN EN 15634-1:2009.



2. Procedure

2.1 Before You Begin

2.1.1 Precautions and Warnings

Detection of hazelnut DNA using the foodproof Hazelnut Detection Kit requires DNA amplification by PCR. The kit provides all required reagents in a ready-to-use Master Mix.

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-resistant pipette tips.
- To avoid carry-over contamination, transfer the required solution volume for all samples in the 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 foodproof Magnetic Preparation Kit III (Product No. KIT230182)
- Real-time PCR instruments with FAM and HEX/VIC detection channels
- 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
- 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 Hazelnut Detection Kit was validated with foods such as cookies, cereals, pastries, chocolates, etc. Food products with high acetic acid concentrations may lead to false-negative results because DNA may become denatured under these conditions.

For preparation of genomic DNA from raw material of plant origin or from food, refer to the corresponding product package insert 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 provided 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 the provided PCR-grade water (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 for your real-time PCR cycler):

Program:

<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

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 Hazelnut 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.

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 to a well. If less than 5 µL, add H₂O to 5 µL.
 - For the negative control, add 5 µL PCR-grade H₂O (vial 3, colorless cap) to a well.
 - For the positive control, add 5 µL Control Template (vial 2, purple cap) to a well.
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 s.
5. Cycle the samples as described above.

2.4 Data Interpretation

The amplification of hazelnut DNA is analyzed in the fluorescence channel FAM and the internal control in channel HEX/VIC.

Result in Channel FAM hazelnut	Result in Channel HEX/VIC Internal Control	Result Interpretation
Positive	Positive/Negative	Positive for hazelnut
Negative	Positive	Negative for hazelnut
Negative	Negative	Invalid

2.5 Related Procedures

2.5.1 Prevention of Carry-over Contamination

The heat-labile Uracil-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 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 Hazelnut Detection Kit, prevention of carry-over contamination is 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 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 program. - 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.
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 precipitate 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 regarding 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 regarding indication of the ingredients present in foodstuffs.

4. Supplementary Information

4.1 Ordering Information

Hygiena Diagnostics is offering 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

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.

4.3 Trademarks

foodproof® is a registered trademark of Hygiena Diagnostics GmbH. Other brand or product names are trademarks of their respective holders.

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 62



5. Change Index

Version 1, October 2014

First version of the package insert.

Version 2, March 2017

License Notice changed.

Revision A, December 2023

Rebranding and new layout.

R 302 62 20 -> INS-KIT230059-REVA



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