For food testing purposes. FOR IN VITRO USE ONLY.

foodproof® Peanut Detection Kit
- 5’ Nuclease -

Version 2, March 2017

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

Order No. R 302 63

Kit for 64 reactions for a maximum of 62 samples

Store the kit at -15 to -25 °C

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1. Product Overview

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 [H_2O PCR-grade (vial 3, colorless cap)] can be analyzed.

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.

Kit Contents

Vial	Label	Contents / Function / Storage
1 yellow cap	**food**proof Peanut Detection Kit - Master Mix -	• 2 x 650 µl • Ready-to-use primer and 5' nuclease probe mix for the amplification of peanut-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	**food**proof Peanut 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	**food**proof Peanut 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 °C to +8 °C for up to one month

Product Description

The **food**proof Peanut Detection Kit provides PCR primers, hydrolysis probes (5' nuclease probes), and convenient premixed reagents for the species-specific amplification and detection of peanut DNA (*Arachis hypogaea*). As even trace amounts of peanut in food can trigger severe immune responses to consumers, multi-copy targets of the peanut genome were used to increase the sensitivity of the PCR-assay. Additionally, the Control Template and H_2O PCR-grade monitor the PCR run for validity.
In combination with the **food**proof Sample Preparation Kit III (S 400 06.1) and the **food**proof Magnetic Preparation Kit III (S 400 13 L), peanut 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.

Application

The **food**proof Peanut Detection Kit is intended for food testing purposes only. Users may identify low amounts of peanut 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 (A 500 13) at www.bc-diagnostics.com

Product Characteristics

Specificity	The primers and hydrolysis probes (5' nuclease probes) provided in the Master Mix, (vial 1, yellow cap) are sequence-specific for peanut and the Internal Control, respectively. Specificity of the assay was proven by 81 plant and animal species, as well as 19 commercial food products.
Sensitivity	The limit of detection was determined to be 0.2 peanut genome equivalent and 1 ppm in peanut 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 peanut in food samples was measured to be below 13.7 % for Allergen RM 800 at 800 ppm, 60 % (0.3ppm ±0.2) for 1 ppm in ice cream, and 53.6 % (0.2ppm ±0.1) for 1 ppm 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 Cycler.

Note: *More detailed information is listed in the Validation Data Report of the **food**proof Peanut Detection Kit. Please contact our Technical Support (bcd@bc-diagnostics.com).*

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 labelled food products. EU Commission Directive 2007/68/EC defines 20 allergenic substances which must be declared if contained in food products, including peanut. 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 in terms of monitoring of production- and cleaning processes or to establish and comply with in-house threshold levels. Therefore, the **food**proof Peanut Detection Kit was designed for both qualitative and quantitative analyses, when used in combination with the Allergen RM 800 reference material (A 500 13). The detection of species which contain allergenic ingredients by molecular methods is regulated by DIN EN 15634-1:2009.

2. Procedure

2.1 Before You Begin

Precautions and Warnings

Detection of peanut DNA using the **food**proof Peanut 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-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 Peanut Master Mix (vial 1, yellow cap) from light, and avoid multiple freezing and thawing cycles.

Additional Equipment and Reagents Required

- Allergen RM 800 (A 500 13) reference material for quantitative purposes
- **food**proof Sample Preparation Kit III (S 400 06.1) or **food**proof Magnetic Preparation Kit III (S 400 13 L)
- 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

Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. The **food**proof Peanut Detection Kit was validated with foods, such as chocolates, cereals, among others. Food products with high acetic acid concentrations may lead to false-negative results because DNA may be 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).

Assay Time

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

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).

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H_2O PCR-grade (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 of 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 **food**proof Peanut 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 (if less than 5 µl add H_2O to 5 µl) to a well.
   - For the negative control, add 5 µl H_2O PCR-grade (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 peanut DNA is analyzed in the fluorescence channel FAM and the internal control in channel HEX/ VIC.

Result in channel FAM Peanut	Result in channel HEX/ VIC Internal Control	Result interpretation
Positive	**Positive/ Negative**	**Positive for peanut**
Negative	**Positive**	**Negative for peanut**
Negative	**Negative**	**Invalid**

2.5 Related Procedures

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 **food**proof Peanut 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

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

- **food**proof Sample Preparation Kit III (S 400 06.1)
- **food**proof Magnetic Preparation Kit III (S 400 13 L)
- Allergen RM 800 (A 500 13)

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 BIOTECON Diagnostics, please contact our Technical Support staff (bcd@bc-diagnostics.com). Our scientists commit themselves to providing rapid and effective assistance. We welcome 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.

5. Change Index

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First version of the package insert.

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License Notice changed.

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