

foodproof® Campylobacter Quantification Kit

Revision B, November 2025

PCR system for the quantitative detection of Campylobacter DNA using real-time PCR instruments.

Product No. KIT230041

Kit for 96 reactions for a maximum of 94 samples Store at −15 to −25 °C

For food testing purposes

FOR IN VITRO USE ONLY





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1. What this Product Does

1.1 Number of Tests

The detection system is designed for 96 reactions with a final reaction volume of 25 μ L each. Up to 94 samples (single sample preparation) plus positive and negative control reactions can be analyzed 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 components as described in the following Kit Contents table.

1.3 Kit Contents

Vial No. /Cap Color	Label	Contents, Function, Storage	
1 yellow cap	foodproof® Campylobacter Master Mix	 3 x 600 μL Ready-to-use primer and hydrolysis probe mix specific for Campylobacter DNA and the Campylobacter-specific Internal Control (IC). For amplification and detection of Campylobacter-specific sequences. Store at -15 to -25 °C. Avoid repeated freezing and thawing! Protect from light! 	
2 red cap	foodproof Campylobacter Enzyme Solution	 3 x 32 μL Contains Taq DNA Polymerase and Uracil-DNA Glycosylase (UNG, heat labile) for prevention of carryover contamination. Store at -15 to -25 °C. 	
3 white cap	foodproof Campylobacter Internal Control	 3 x 32 μL Contains a stabilized solution of plasmid DNA and a yellow dye for better visualization. For use as an internal amplification control. Store at -15 to -25 °C. After first thawing, store at 2 to 8 °C for up to one month. 	
4 purple cap	foodproof Campylobacter Quantification Standard	 1 x 180 μL Contains a stabilized solution of plasmid DNA. For use as a PCR positive control. For use as a standard for <i>Campylobacter</i> quantification (10⁶ CFU/reaction). Store at -15 to -25 °C. After first thawing, store at 2 to 8 °C for up to one month. 	
5 colorless cap	H₂O, PCR-grade	 1 x 1 mL Nuclease-free, PCR-grade H₂O. For use as a PCR negative control. Store at -15 to -25 °C. 	
6 blue cap	foodproof Campylobacter Dilution Buffer	 3 x 1 mL For dilution of the Quantification Standard. Store at -15 to -25 °C. 	



1.4 Additional Equipment and Reagents Required

- Real-time PCR instruments with FAM, VIC/HEX, ROX and Cy5 detection channels
- Real-time PCR compatible tubes, strips or plates with optical cap or foil applicable for the PCR cycler used
- Sample preparation kit: foodproof StarPrep One Kit (Product No. KIT230175)
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Sterile reaction tubes for preparing PCR mixes and dilutions

1.5 Applicability Statement

The foodproof *Campylobacter* Quantification Kit is intended for the rapid detection of DNA from thermotolerant *Campylobacter* spp. isolated from enrichment cultures or rinse samples prepared by valid methods and inoculated with all kinds of foods that are potentially contaminated with thermotolerant *Campylobacter* spp. The kit detects the following species:

foodproof Campylobacter Quantification Kit		
Thermotolerant Campylobacter spp.		
C. jejuni		
C. coli		
C. lari		
C. upsaliensis		
Other Campylobacter spp.		
C. insulaenigrae		

This detection kit must not be used in diagnostic procedures.

This kit has been developed for real-time PCR instruments with FAM, VIC/HEX, ROX and Cy5 detection channels. The performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480 (Roche Diagnostics), 7500 Real-Time PCR System (Applied Biosystems), Mx3000P® QPCR System (Stratagene) and Rotor-Gene® 6000 (Qiagen).

Note:

A Color Compensation Set (Color Compensation Set 3; Product No. KIT230005) is necessary and will be supplied by Hygiena® Diagnostics for users of the LightCycler 480 Systems I and II. Contact Hygiena Diagnostics for further information.



2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of *Campylobacter* DNA using the foodproof *Campylobacter* Quantification Kit requires DNA amplification by PCR. The detection kit provides all the reagents required for the PCR. To achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carryover- or cross-contamination:

- Prepare appropriate aliquots of the 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 carryover 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 carryover contamination. Use a PCR hood for all pipetting steps.

Keep the foodproof Campylobacter Master Mix (vial 1, yellow cap) away from light.

2.1.2 Waste Disposal

Place any waste and biohazard material potentially contaminated with pathogenic bacteria in an appropriate plastic Contaminated Waste bag and label as follows: CONTAMINATED Waste, Room number, date and initials. The bag should be autoclaved and then disposed of according to local regulations.

2.1.3 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 or from food enrichments, refer to the corresponding product package inserts of a suitable sample preparation kit (see Additional Equipment and Reagents Required).

2.1.4 Enrichment

For qualitative purposes, enrichment broth and temperature can be applied according to DIN EN ISO 10272-1:2006, BAM (Chapter 7) or USDA. Other suitable, validated enrichment procedures can also be used.

2.1.5 Rinse Sample

For quantification purposes, rinse samples and initial suspensions of poultry or meat with buffered peptone water can be prepared according to DIN EN ISO 6887.

2.1.6 DNA Extraction

Hygiena Diagnostics provides sample preparation kits suitable for all kinds of foods and raw materials (see "Additional Equipment and Reagents Required"). For more product information, visit www.hygiena.com.



2.1.7 Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [foodproof *Campylobacter* Quantification Standard (vial 4, purple cap)] or with a positive sample preparation control. In case of a quantitative detection using an external standard, the undiluted foodproof Quantification Standard (vial 4, purple cap) serves as positive control for all three detection channels (FAM, ROX and Cy5).

2.1.8 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 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 Procedure

2.2.1 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):

<u>Pre-incubation</u>	1 cycle
Step 1: Step 2:	37°C for 4 minutes 95°C for 5 minutes
<u>Amplification</u>	50 cycles
Step 1:	95°C for 5 seconds

^{*} Fluorescence detection in step 2

For some real-time PCR instruments, the type of probe quencher as well as the use of a passive reference dye must be specified. The foodproof Campylobacter Quantification Kit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.

Note: For users of the Agilent Mx3005P instrument: Click 'Instrument \rightarrow Filter Set Gain Settings' to open the Filter Set Gain Settings dialog box. For FAM, modify the Filter Set Gain Setting to 'x1'.

2.2.2 Procedure A: Quantitative Detection using External Standards

Each individual run consists of the following:

- Six dilutions (in duplicate) of the foodproof *Campylobacter* Quantification Standard (vial 4, purple cap) to generate the respective standard curve (see table below),
- A variable number of sample preparations to be analyzed for Campylobacter DNA amplification,
- At least one negative control reaction as a control for contamination of the PCR Master Mix.

Therefore, a typical experiment consists of 13 reactions for controls, plus n x reactions needed for the samples of interest, where (n) indicates the number of food samples of interest.



Note: Since 96 reactions can be made with the kit, up to 83 food samples can be analyzed quantitatively during one PCR run.

2.2.3 Dilution of Quantification Standard

Quantification of the *Campylobacter* content via the external standard (standard curve) procedure requires the stepwise dilution of the foodproof *Campylobacter* Quantification Standard (vial 4, purple cap) with the foodproof *Campylobacter* Dilution Buffer (vial 6, blue cap) as shown below.

Prepare each dilution step with a final volume of 100 μ L by using 10 μ L of the previous dilution step and 90 μ L of the foodproof *Campylobacter* Dilution Buffer (vial 6, blue cap).

Dilution Step	Dilution	Concentration Entered as Standard (CFU/reaction)
1	Undiluted	1,000,000
2	1:10	100,000
3	1:100	10,000
4	1:1,000	1,000
5	1:10,000	100
6	1:100,000	10

2.2.4 Procedure B: Qualitative Detection

Each individual run consists of the following:

- A variable number of sample preparations to be analyzed for Campylobacter DNA amplification,
- At least one negative control reaction as a control for contamination of the PCR Master Mix,
- At least one positive control reaction. To prepare a positive control, replace the template DNA with the
 provided control DNA [foodproof *Campylobacter* Quantification Standard (vial 4, purple cap)] or with a
 positive sample preparation control.

Therefore, a typical experiment consists of 2 reactions needed for controls, plus n x reactions needed for the samples of interest, where (n) indicates the number of food samples of interest.

Note: Since 96 reactions can be made with the kit, up to 94 food samples can be analyzed qualitatively during one PCR run.





2.2.5 Preparation of the PCR Mix

Proceed as described below to prepare a 25 µL standard reaction.

Always wear gloves when handling the PCR vessels.

- 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 reaction tube (0.5 2.0 mL) depending on the number of reactions, prepare the PCR Mix by adding the following components in the order listed below:

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 to be cycled, plus one or two additional reactions to cover pipetting losses.

Component	Volume (μL)
foodproof Campylobacter Master Mix (vial 1, yellow cap)	18.0
foodproof Campylobacter Enzyme Solution (vial 2, red cap)	1.0
foodproof Campylobacter Internal Control (vial 3, white cap)	1.0
Total volume	20.0

3. Prepare reaction mixtures:

- Mix carefully but thoroughly by pipetting up and down. Do not vortex.
- Pipet 20 μL of PCR mix into each PCR vessel.
- For the samples of interest, add 5 μL of sample DNA.
- For the negative control, add 5 μL of H₂O, PCR-grade (vial 5, colorless cap).
- Procedure A: For the external standards, add 5 μL of each dilution of foodproof Campylobacter Quantification Standard (vial 4, purple cap) to the PCR vessel.
- Procedure B: For the positive control, add 5 μL of foodproof Campylobacter Quantification Standard (vial 4, purple cap) to the PCR vessel.
- 4. Seal the PCR vessels accurately with optical caps or foil.
- 5. Briefly spin the PCR vessels in a suitable centrifuge.
- 6. Cycle the samples as described above.

2.3 Data Interpretation

General remarks 2.3.1

The amplification of DNA from thermotolerant Campylobacter species C. jejuni, C. coli, C. lari and C. upsaliensis is analyzed in the fluorescence channel suitable for FAM-labeled probe detection. In addition, C. jejuni can be identified in the ROX channel. An identification of C. coli is accomplished in the channel suitable for Cy5-labeled probes. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for VIC/HEX.



2.3.2 Procedure A – Quantification using External Standards

Define the positions of the dilutions of the foodproof *Campylobacter* Quantification Standard as "Standard" with the respective concentrations given in the table above to generate a standard curve. Alternatively, a given standard curve from a previous PCR run can be imported if the real-time PCR instrument allows.

The foodproof *Campylobacter* Quantification Standard is defined as CFU/reaction. The use of the calibration curve results in one value for every sample analyzed. This value can be converted to CFU/mL according to the following equation:

result $[CFU/mL] = (result [CFU/reaction] \times elution volume [\mu L]) / (sample volume [\mu L]) \times total test volume [mL])$

- elution volume = final volume after sample preparation
- sample volume = volume used per PCR reaction
- total test volume = initial volume used from the enrichment culture or rinse sample for PCR preparation

Example:

The following calculation is suitable for samples prepared with the foodproof StarPrep One Kit:

- elution volume = 200 μL
- sample volume = 5 μL
- total test volume = 0.1 mL

result [CFU/mL] = (result [CFU/reaction] x 200 μ L) / (5 μ L x 0.1 mL) = result [CFU/reaction] x 400 [reaction/mL]

For the conversion of results from, e.g., rinse samples from CFU/mL into CFU/g, the following calculation can be applied:

result $[CFU/q] = (result [CFU/mL] \times total sample volume [mL]) / sample amount [q]$

- total sample volume = total volume of the rinse sample
- sample amount = amount of the food sample used to prepare the rinse sample



2.3.3 Procedure B – Qualitative Detection

For qualitative detection, compare the results from all channels FAM (thermotolerant *Campylobacter* spp.), ROX (*Campylobacter jejuni*), Cy5 (*Campylobacter coli*) and VIC/HEX (Internal Control) for each sample, and interpret the results as described in the following table.

Thermotolerant Campylobacter spp.	Campylobacter jejuni	Campylobacter coli	Internal Control	Result Interpretation
FAM Channel	ROX Channel	Cy5 Channel	VIC/HEX Channel	
Positive	Negative	Negative	Positive or Negative	Positive
Positive	Positive	Negative	Positive or Negative	Positive (<i>C. jejuni</i>)
Positive	Negative	Positive	Positive or Negative	Positive (<i>C. coli</i>)
Positive	e Positive Positive		Positive or Negative	Positive (<i>C. jejuni</i> and <i>C. coli</i>)
Negative	Negative	Negative	Positive	Negative
Negative	Negative	Negative	Negative	Invalid

For samples with a very low amount of *Campylobacter* DNA (crossing point > 33 in the FAM channel), identification of *C. jejuni* (ROX channel) and *C. coli* (Cy5 channel) might not be possible.





3. Troubleshooting

Observation	Possible Reason	Recommendation		
No. 25 and	Incorrect detection channel has been chosen.	• Set Channel settings to FAM, VIC/HEX, ROX and Cy5.		
No signal increase is observed, even with positive controls.	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.		
No signal increase in the VIC/HEX channel.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	 Use a 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, substituting with H₂O, PCR-Grade). 		
	Inappropriate storage of kit components.	 Store the foodproof Campylobacter Master Mix (vial 1, yellow cap) at -15 to -25 °C, protected from light. Avoid repeated freezing and thawing. 		
Fluorescence intensity is too low.	foodproof <i>Campylobacter</i> Master Mix (vial 1, yellow cap) is not homogeneously mixed.	Mix the foodproof <i>Campylobacter</i> 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.	Carryover contamination is present.	 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 carryover contamination. Add positive controls after sample and negative control reaction vessels have been sealed. 		
Fluorescence	Insufficient centrifugation of the PCR vessels. Prepared PCR mix is still in the upper part of the vessel.	Always centrifuge reaction vessels.		
intensity varies.	Outer surface of the vessel or seal is dirty (e.g., by direct skin contact).	Always wear gloves when handling the vessel and seal.		





4. Additional Information on this Product

4.1 How this Product Works

The foodproof Campylobacter Quantification Kit provides primers and hydrolysis probes (for sequence-specific detection), convenient premixed reagents and a control template for reliable interpretations of results. 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 3, white cap). The IC must be added to each reaction. The hydrolysis probe was designed to bind specifically to the IC, allowing detection in the VIC/HEX channel, whereas the Campylobacter DNA is detected in the FAM, ROX and Cy5 channels.

In cases 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 *Campylobacter* DNA in the sample.

The foodproof Campylobacter Quantification Kit minimizes contamination risk and contains all reagents needed for detection of Campylobacter DNA. Primers and probes provide specific detection of Campylobacter DNA in food samples. This kit has been developed for real-time PCR instruments with FAM, VIC/HEX, ROX and Cy5 detection channels.

4.2 Test Principle

- 1. Using the supplied sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and its associated reagents amplify and simultaneously detect fragments of Campylobacter genomic DNA.
- 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 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.

4.3 Prevention of Carryover Contamination

The heat-labile Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover 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 dUTP 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 Campylobacter 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 Campylobacter Quantification Kit, decontamination can be achieved with the provided reagents.



4.4 Background Information

The genus *Campylobacter* is a group of spiral-shaped bacteria that currently comprises 22 species currently. Most human illness is caused by one species, *Campylobacter jejuni*, but 1–10% of human *Campylobacter* cases are caused by other species (e.g., *C. coli* or *C. lari*). In Germany in 2007, *Campylobacter* was the most frequent bacterium (even more frequent than *Salmonella*), causing food-related diarrhea [1]. While *Salmonella* is commonly known as a foodborne pathogen, many people do not know about *Campylobacter*. The symptoms of *Campylobacter* infection are similar to Salmonellosis. After an incubation time of 3–5 days, the patient gets diarrhea, nausea, cramps, headache, fever and insomnia. In most cases, bacteria are transmitted by food (mainly raw poultry and raw milk products) or surface water contaminated with feces. Since conventional microbiological methods for the detection and identification of *Campylobacter* are very time-consuming, PCR has been introduced to the food industry as a highly sensitive and specific detection method [2].

4.5 Product Characteristics

Specificity: The foodproof *Campylobacter* Master Mix is sequence-specific for the most important members of the genus *Campylobacter*. Inclusivity has been tested with 193 strains of the 4 species detected by this kit, and all of them could be detected (100% inclusivity). Strains of *Campylobacter* species other than the four target organisms might be detected (e.g., *C. insulaenigrae*). Exclusivity was determined using 74 species of the genus *Campylobacter* (*C. concisus*, *C. curvus*, *C. fetus*, *C. gracilis*, *C. helveticus*, *C. hominis*, *C. hyointestinalis*, *C. mucosalis*, *C. rectus*, *C. showae*, *C. sputorum*), phylogenetically closely related bacterial strains (*Helicobacter* and *Arcobacter*) and strains of the same microbiological environment.

Sensitivity: A relative detection limit of 1 to 10 cells per 25 g of sample can be achieved with all kinds of foods. The foodproof *Campylobacter* Quantification Kit detects down to $10^3 - 10^4$ CFU/mL of enrichment cultures and quantification is possible down to 10^3 CFU/mL in rinse samples of poultry and meat prepared with buffered peptone water (depending on the sample preparation kit used, see "Additional Equipment and Reagents Required").

4.6 References

- 1. Robert Koch Institute. (2008) Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2007. Berlin, Germany.
- 2. Scheu PM, Berghof K, Stahl U. (1998) Detection of pathogenic and spoilage micro-organisms in food with the polymerase chain reaction. *Food Microbiology* 15, 13 31.

4.7 Quality Control

The foodproof Campylobacter Quantification Kit is function tested using the LightCycler 480 System I and II.



5. Supplementary Information

5.1 Ordering Information

In addition to the foodproof *Campylobacter* Quantification Kit, Hygiena Diagnostics offers a broad range of reagents and services. For a complete overview and for more information, visit us at www.hygiena.com and contact us via email or phone.

5.2 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 Hygiena Diagnostics GmbH. Hygiena® is a trademark of Hygiena. 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 Hygiena Diagnostics, contact our Technical Support staff (for details see www.hygiena.com/support). Our scientists commit themselves to providing rapid and effective help. 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.

5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 302 05

6. Change Index

Version 1

First version of the package insert.

Version 2, December 2008

New product name extension: 5'Nuclease.

Version 3, August 2011

Page 8: Note for users of the Agilent Mx3005P instrument added.

Version 4, March 2017

License Notice changed.



Product Instructions



Revision A, September 2023
Rebranding and new layout. Change of company name and product number.
R 302 05 20 -> INS-KIT230041-REVA.

Revision B, November 2025 Updated sample preparation kit options and quantification example.





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