foodproof® *Brucella* Detection Kit

Revision A, January 2024

PCR system for the qualitative detection of *Brucella* DNA using real-time PCR instruments.

Product No. KIT230040

PCR system for 96 reactions for a maximum of 94 samples

Store the PCR kit at -15 to -25 °C

For food testing purposes.

FOR *IN VITRO* USE ONLY

Table of Contents

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 µL 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/Cap Color	Label	Contents / Function / Storage
1 yellow cap	foodproof *Brucella* Master Mix	• 3 x 600 µL • Ready-to-use primer and Hydrolysis Probe mix specific for *Brucella* DNA and the *Brucella*-specific Internal Control (IC). • For amplification and detection of *Brucella*-specific sequences. • Store at -15 to -25°C. • **Avoid repeated freezing and thawing! Protect from light!**
2 red cap	foodproof *Brucella* Enzyme Solution	• 3 x 32 µL • Contains Taq DNA Polymerase and Uracil-DNA Glycosylase (heat labile) for prevention of carry-over contamination. • Store at -15 to -25°C.
3 white cap	foodproof *Brucella* Internal Control	• 3 x 32 µL • Contains a stabilized solution of plasmid DNA. • 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 *Brucella* Control Template	• 1 x 50 µL • Contains a stabilized solution of plasmid DNA. • For use as a PCR run positive control. • Store at -15 to -25°C. • After first thawing store at 2 to 8 °C for up to one month.
5 colorless cap	H_2O, PCR-grade	• 1 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 Additional Equipment and Reagents Required

- Real-time PCR instruments with a FAM, VIC/HEX, ROX/Texas Red and Cy5 detection channel
- Real-time PCR compatible tubes, strips or plates with optical cap or foil applicable for the PCR cycler in use
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Sterile reaction tubes for preparing PCR mixes and dilutions

1.5 Applicability Statement

The foodproof *Brucella* Detection Kit is intended for the rapid detection of *Brucella* spp., including the identification of *Brucella abortus* and *Brucella melitensis.*

The detection kit must not be used in diagnostic procedures.

The kit described in this Instruction Manual has been developed for real-time PCR instruments with FAM, VIC/HEX, ROX/Texas Red and Cy5 detection channels. The performance of the kit was tested with the following real-time PCR instruments: Mx3000p® QPCR System (Stratagene), Mx3005 p® QPCR System (Stratagene), LightCycler® 480 (Roche Diagnostics), Rotor-Gene® 6000 (Corbett Life Science) and iQ5™ (Bio-Rad Laboratories).

Note:
A Color Compensation (Color Compensation Set 3; Product No. KIT230005) is necessary and will be supplied by Hygiena Diagnostics for users of the LC 480 Systems I and II. Please contact Hygiena Diagnostics for further information.

2. How to Use this Product

2.1 Before You Begin

2.1.1 Precautions

Detection of *Brucella* DNA using the foodproof *Brucella* Detection Kit requires DNA amplification by PCR. The detection kit provides all the reagents required for the PCR. In order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. Follow the instructions below to avoid nuclease-, carry-over-, 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 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.

Keep the foodproof *Brucella* 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.

2.1.4 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 *Brucella* Control Template (vial 4, purple cap)] or with a positive sample preparation control.

2.1.5 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 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.

Pre-incubation	**1** cycle
Step 1:	40 °C for 2 minutes
Step 2:	95 °C for 10 minutes
Amplification	**45** cycles
Step 1:	95 °C for 15 seconds
Step 2*:	63 °C for 30 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 specified. The foodproof *Brucella* Detection Kit contains probes with a non-fluorescent (“dark”) 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’.

2.2 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. 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 mentioned below:

   Note: 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
foodproof *Brucella* Master Mix (vial 1, yellow cap)	18.0 µL
foodproof *Brucella* Enzyme solution (vial 2, red cap)	1.0 µL
foodproof *Brucella* Internal Control (vial 3, white cap)	1.0 µL
Total volume	20.0 µL

3. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
   - Pipet 20 µL PCR mix into each PCR vessel.
   - For the samples of interest, add 5 µL sample DNA.
   - For the negative control, add 5 µL H_2O, PCR-grade (vial 5, colorless cap).
   - For the positive control, add 5 µL foodproof *Brucella* Control Template (vial 4, purple cap).
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

The amplification of DNA of *Brucella abortus* is analyzed in the fluorescence channel suitable for FAM-labeled probes detection and of *Brucella melitensis* in the detection channel for VIC/HEX. The amplification of DNA of any member of the genus *Brucella* is analyzed in the fluorescence channel suitable for ROX/Texas Red-labeled probe detection. The amplification signal of the Positive Control can be detected in all three channels. The specific amplification of the Internal Control (IC) is analyzed in the fluorescence channel suitable for Cy5. Compare the results from channel FAM (*Brucella abortus*), VIC/HEX (*Brucella melitensis*), ROX/Texas Red (*Brucella*), and channel Cy5 (Internal Control) for each sample and interpret as described in the following table.

Channel FAM	Channel VIC/HEX	Channel ROX	Channel Cy5 (IC)	Result Interpretation
Positive	negative	Positive	Positive or Negative	Positive for *Brucella* and *B. abortus*
Negative	positve	Positive	Positive or Negative	Positive for *Brucella* and *B. melitensis*
Positive	positive	Positive	Positive or Negative	Positive for *Brucella, B. abortus* and *B. melitensis*
Negative	Negative	Positive	Positive or Negative	Positive for *Brucella* (non *B. abortus* or *B. melitensis*)
Negative	Negative	Negative	positive	Negative for *Brucella, B. abortus* or *B. melitensis* respectively
Negative	Negative	Negative	Negative	Invalid

Note: A prerequisite for the unambiguous discrimination of *Brucella abortus* (FAM), *Brucella melitensis* (VIC/HEX), *Brucella* (ROX/TEXAS Red) and Internal Control DNA in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM, VIC/HEX, ROX/Texas Red and Cy5. Please refer to the operation manual for your real-time PCR cycler for further information.

3. 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, VIC/HEX, ROX/Texas Red or Cy5.
	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 channel Cy5.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	• Dilute samples or pipet a lower amount of sample DNA (e.g., 2.5 µL instead of 5 µL, substitute with H_2O, PCR-Grade).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the foodproof *Brucella* Master Mix (vial 1, yellow cap) at -15 to -25 °C, protected from light. • Avoid repeated freezing and thawing.
	foodproof *Brucella* Master Mix (vial 1, yellow cap) is not homogeneously mixed.	• Mix the foodproof *Brucella* 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. • Add positive controls after sample and negative control reaction vessels have been sealed.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR vessels. Prepared PCR mix is still in the upper part of the vessel.	• Always centrifuge reaction vessels.
	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 *Brucella* Detection 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 has to be added to each reaction. A Hydrolysis Probe was designed to bind specifically to the IC, allowing detection in the Cy5 channel, whereas the *Brucella* DNA is detected in the FAM (*B. abortus*), VIC/HEX (*B. melitensis*) and ROX/Texas Red (genus *Brucella*) channel. In case of a negative result due to inhibition of amplification by the sample DNA of interest, the amplification of the IC is suppressed as well. A negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of *Brucella* DNA in the sample. The foodproof *Brucella* Detection Kit minimizes contamination risk and contains all reagents needed for detection of *Brucella* DNA. Primers and probes provide specific detection of *Brucella* DNA in food samples. The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM, VIC/HEX, ROX/Texas Red and Cy5 detection channel.

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 *Brucella* 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 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 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 *Brucella* 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 *Brucella* Detection Kit, decontamination can be achieved with the provided reagents.

4.4 Background Information

The genus *Brucella*, member of the *Brucellaceae* family (class: *Alphaproteobacteria*), comprises the six species *B. abortus, B. melitensis, B. suis*, *B. ovis*, *B. canis,* and *B. neotomae*. Bacteria of the genus *Brucella* are gram-negative, facultative intracellular, non-motile, non-spore forming pathogens that infect a wide range of animal hosts and the species *B. abortus, B. melitensis, B. suis*, and *B. canis* may also infect humans. *Brucella* sp. are the causative organisms of the disease brucellosis in humans, also called undulant fever, or Malta fever.

Brucellosis is a highly contagious zoonosis (infectious disease transmitted from animals to humans) caused by eating raw, minced meat or contaminated or untreated milk (and its derivates) or through direct contact with infected animals, which may include dogs, pigs, camels and ruminants, primarily sheep, goats, cattle, bison.

Common microbiological and serological methods for the detection and identification of *Brucella* spp. are very time-consuming and very hazardous for the laboratory staff [1, 2]. Therefore, several PCR ELISA and real-time PCR assays were developed to fulfill the need for rapid, sensitive and specific systems for the detection of *Brucella* spp. [3].

4.5 Product Specifications

Specificity:

The foodproof *Brucella* Master Mix is sequence-specific for highly conserved genes found in all *Brucella* sp., in *Brucella abortus*, or *Brucella melitensis*. Inclusivity has been tested with more than 50 strains of *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae,* where all of them could be detected (100% inclusivity). Exclusivity was determined using 25 species of closely related organisms or organisms occurring in the same habitat.

4.6 References

1. Al Dahouk S., *et al.* (2003) Laboratory-based diagnostics of brucellosis – a review of the literature. Part I: techniques for direct detection and identification of *Brucella* spp. *Clin Lab* 49, 487-505.
2. Al Dahouk S., *et al.* (2003) Laboratory-based diagnostics of brucellosis – a review of the literature. Part II: serological tests for brucellosis. *Clin Lab* 49, 577-589.
3. Al Dahouk S., *et al.* (2004) The detection of *Brucella* spp. Using PCR-ELISA and real-time PCR assays. *Clin Lab* 50, 387-394.

4.7 Quality Control

The foodproof *Brucella* Detection Kit is function tested using the LightCycler 480 System.

5. Supplementary Information

5.1 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.

5.2 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

5.3 Trademarks

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5.4 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.

5.5 Reference Number

The reference number and original Hygiena Diagnostics GmbH article number: R 302 04

6. Change Index

Version 1
First version of the package insert.

Version 2
New product name extension: 5’ Nuclease.

Version 3, July 2010
Page 7: Note for users of the Agilent Mx3005p instrument added.

Version 4, March 2017
License Notice changed.

Revision A, January 2024
Rebranding and new layout.
R 302 04 20 -> INS-KIT230040-RevA

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