

FOR *IN VITRO* USE ONLY

foodproof[®] *Listeria* Genus Detection LyoKit – 5´Nuclease –

Version 1, May 2017

PCR kit for the qualitative detection of *Listeria* sensu stricto species (*L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. innocua* and *L. marthii*), using real-time PCR instruments.

Order No. R 602 20-1 / R 602 20-2

**Kit for 96 reactions (lyophilized) for a maximum of
94 samples**

Store the kit at 2 to 8 °C

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1. What this Product Does

Number of Tests

The kit 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.

Storage and Stability

- Store the kit at 2 °C to 8 °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

Component	Label	Contents / Function / Storage
foodproof® <i>Listeria</i> Genus Detection LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing a 8-tube strip mat • R 602 20-1 with white low profile tubes* • R 602 20-2 with clear regular profile tubes*	<ul style="list-style-type: none"> • 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for <i>Listeria sensu stricto</i> DNA and the Internal Control (IC) as well as Taq DNA Polymerase and Uracil-DNA Glycosylase (UNG, heat labile) for prevention of carry-over contamination. • For amplification and detection of <i>Listeria sensu stricto</i> specific sequences. • Store at 2 °C to 8 °C in the aluminum bag (sealed and containing silica gel pads). • Protect from light and moisture!
Control Template	Vial 2 (purple cap)	<ul style="list-style-type: none"> • 1 x 250 µl • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.
H ₂ O PCR-grade	Vial 3 (colorless cap)	<ul style="list-style-type: none"> • 2 x 1 ml • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	<ul style="list-style-type: none"> • 12 x 8-cap strip • For use in real-time PCR after addition of samples.

*Tube profile and instrument compatibility chart is available online: www.bc-diagnostics.com/compatibility-chart

Additional Equipment and Reagents Required

- Real-time PCR cyclers suitable for detection of FAM- and VIC/Yakima Yellow-labeled probes as well as for using low or regular profile strip tubes. In case the strip tubes don't fit for the instrument, the samples should be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.
- Sample Preparation Kit
 - foodproof® StarPrep Two Kit (Order No. S 400 08)¹
 - foodproof® StarPrep Two 8-Strip Kit (Order No. S 400 17 L)¹
 - foodproof® ShortPrep II Kit (Order No. S 400 02)¹ **or**
 - foodproof® Magnetic Preparation Kit II (Order No. S 400 12 L)¹

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
and optionally
- Vortex centrifuge Multispin MSC-6000 for PCR-strips (Order No. D 110 66)¹ **with**
- SR-32, Rotor for MSC-3000/6000 (Order No. D 110 65)¹ **or**
- Vortex centrifuge CVP-2 for PCR-plates (Order No. D 110 67)¹

¹ Available from BIOTECON Diagnostics; see ordering information for details

Applicability Statement

The foodproof® *Listeria* Genus Detection LyoKit – 5'Nuclease – is intended for the rapid detection of *Listeria sensu stricto* species isolated from enrichment cultures prepared by valid methods and inoculated with all relevant kinds of foods and environmental samples that are potentially contaminated with *Listeria*. The kit must not be used



in diagnostic procedures. The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM and a VIC/Yakima Yellow or HEX detection channel. The performance of the kit was tested with the following real-time PCR instruments: LightCycler[®] 480, LightCycler[®] 96 (Roche Diagnostics), Mx3005P[®], AriaMx (Agilent Technologies), ABI 7500 fast (Thermo Fisher) and PikoReal[®] 24 (Thermo Fisher).

Note: When testing the samples on a LightCycler 480 instrument, it is recommended to carry out the DNA extraction with a wash step (**foodproof**[®] StarPrep Two Kit, S 400 08.1, Procedure A: STANDARD protocol).

2. How to Use this Product

2.1 Before You Begin

Precautions

Detection of *Listeria sensu stricto* DNA using the **foodproof**[®] *Listeria* Genus Detection LyoKit requires DNA amplification by PCR. The kit provides all reagents required for the PCR. However, 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:

- Keep the kit components 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[®] *Listeria* Genus Detection lyophilized PCR mix away from light and moisture.**

Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For preparation of genomic DNA from various sample enrichments, refer to the corresponding product package inserts of a suitable sample preparation kit (see “*Additional Equipment and Reagents Required*”).

DNA Extraction

BIOTECON Diagnostics provides sample preparation kits suitable for all kind of food and environmental samples (see “*Additional Equipment and Reagents Required*”).

For more product information please refer to www.bc-diagnostics.com.

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**[®] *Listeria* Genus Detection Control Template (vial 2, purple cap)] or with a positive sample preparation control.

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H₂O PCR-grade (vial 3, 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.

6. Mix thoroughly using a vortex centrifuge.

Note: BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-3000 (D 110 64) for PCR-strips or vortex centrifuge CVP-2 for PCR-plates (D 110 67). Dedicated protocols are available for this centrifuge.

Note: Alternatively resuspend the pellet by manual mixing. This may be achieved by cautiously pipetting the sample up and down multiple times during step 4 or flipping the tube strips after sealing while pressing down the cap strip.

7. Spin the PCR tube strips for 30 seconds at 150 – 200 g in a suitable centrifuge.

Note: If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1,000 g!

8. Place the samples in your PCR cycler and run the program as described above.

Note: For using any LightCycler 480 instrument, a special adapter (Order No. Z 100 24) is necessary. For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example two strips can be placed in column 1 and 12.

2.3 Data Interpretation

The amplification of the *Listeria* sensu stricto-specific DNA region is analyzed in the fluorescence channel suitable for FAM labeled probes detection. The specific amplification of the Internal Control is analyzed in the fluorescence channel suitable for VIC/YY labeled probes.

Compare the results from channel FAM (*Listeria* sensu stricto) and channel VIC/YY (Internal Amplification Control) for each sample, and interpret the results as described in the table below.

Channel FAM	Channel VIC/YY	Result Interpretation
Positive	Positive or Negative	Positive for <i>Listeria</i> sensu stricto
Negative	Positive	Negative for <i>Listeria</i> sensu stricto
Negative	Negative	Invalid

Note: A prerequisite for the unambiguous discrimination of *Listeria* and the Internal Control DNA in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM and VIC/YY. Please refer to the operation manual of 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.	<ul style="list-style-type: none"> Set Channel settings to FAM or VIC/YY.
	Pipetting errors.	<ul style="list-style-type: none"> Check for correct reaction setup. Repeat the PCR run. Always run a positive control along with your samples.
	No data acquisition programmed.	<ul style="list-style-type: none"> Check the cycle programs.
No signal increase in channel VIC/YY is observed.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	<ul style="list-style-type: none"> Use the recommended DNA sample preparation kit to purify template DNA. Dilute samples or pipet a lower amount of sample DNA (e.g., 5 µl instead of 25 µl).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> Store the foodproof[®] <i>Listeria</i> Genus Detection lyophilized PCR mix at 2 °C to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	<ul style="list-style-type: none"> Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline.	Resuspension of lyophilized PCR mix not complete.	<ul style="list-style-type: none"> Always resuspend lyophilized PCR mix thoroughly.
Negative control samples are positive.	Carry-over contamination.	<ul style="list-style-type: none"> 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 strips. Resuspend PCR mix is still in the upper part of the vessel.	<ul style="list-style-type: none"> Always centrifuge PCR strips.
	Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).	<ul style="list-style-type: none"> Always wear gloves when handling the vessels and seal.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	<ul style="list-style-type: none"> Store the lyophilized PCR mix always in the aluminum bag with the silica gel pad Open strip shortly before filling.

4. Additional Information on this Product

How this Product Works

The **foodproof**[®] *Listeria* Genus Detection LyoKit provides all necessary reagents and a control template for reliable interpretations of results. To ensure maximum reliability of the kit and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is included. A hydrolysis probe was designed to bind specifically the IC, allowing detection in the VIC/Yakima Yellow channel, whereas the *Listeria*-DNA is detected in the FAM channel. In case of a negative result due to inhibition of the 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 *Listeria* sensu stricto in the sample. The **foodproof**[®] *Listeria* Genus Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of *Listeria*-DNA. Primers and probes provide specific detection of *Listeria* sensu stricto-DNA in food and environmental samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only.



Test Principle

1. Using the kit's sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify fragments of specific sequences for *Listeria sensu stricto* species.
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 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 PCR instrument measures the emitted fluorescence of the reporter dye.

Prevention of Carry-Over Contamination

The heat-labile Uracil-DNA N-Glycosylase (UNG) is suitable for preventing carry-over contamination between PCR's. 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 *Listeria* 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**[®] *Listeria* Genus Detection LyoKit, decontamination can be achieved with the provided reagents.

Background Information

The genus *Listeria* currently includes 17 species, *Listeria monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. marthii*, *L. innocua*, *L. grayi*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia*, and *L. booriae*, characterized as gram-positive rod-shaped bacteria. Only *L. monocytogenes* causes Listeriosis, a severe human disease which causes approx. 160 deaths per year alone in the USA [1]. Most susceptible persons are pregnant women, neonates, the elderly and immunosuppressed persons.

Based on the relationship to *L. monocytogenes*, the genus *Listeria* can be divided in *Listeria sensu stricto* (*strictu*), including the species *L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. welshimeri*, *L. marthii* and *L. innocua* and in *Listeria sensu lato* including the species *L. grayi*, *L. fleischmannii*, *L. floridensis*, *L. aquatica*, *L. newyorkensis*, *L. cornellensis*, *L. rocourtiae*, *L. weihenstephanensis*, *L. grandensis*, *L. riparia*, and *L. booriae*. With the exception of *L. grayi*, all *Listeria sensu lato* species were described in the last years [2].

L. monocytogenes is often found in samples that contain other *Listeria* spp.. Therefore detection of *Listeria* species is often used as an indicator to detect conditions that allow for presence, growth, and persistence of *L. monocytogenes*. At least for some of the new *sensu lato* species the suitability as such an indicator is doubtful, because some of this species do not grow at refrigeration temperatures. In addition, it has been proposed that the *Listeria sensu lato* species should be reclassified in new genera. The **foodproof**[®] *Listeria* Genus Detection LyoKit therefore only detects the *Listeria sensu stricto* species.

References

1. Centers for Disease Control and Prevention – Listeriosis <http://www.cdc.gov>.
2. Orsi RH and Wiedmann M. 2016. Characteristics and distribution of *Listeria* spp., including *Listeria* species newly described since 2009. *Appl Microbiol Biotechnol* 100, 5273–5287

Quality Control

The **foodproof**[®] *Listeria* Genus Detection LyoKit is function tested using the LightCycler[®] 480 System.



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5. Supplementary Information

5.1 Ordering Information

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

5.2 License

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 BIOTECON Diagnostics GmbH.

Other brand or product names are trademarks of their respective holders.

5.4 Contact and Support

If you have questions about this or any other product of BIOTECON Diagnostics, please contact our Technical Support staff (for details see www.bc-diagnostics.com). 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.

6. Change Index

First version of the package insert.

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