foodproof® *Listeria monocytogenes* Detection LyoKit – 5´Nuclease –

Version 3, December 2019

PCR kit for the qualitative detection of *Listeria monocytogenes*, using real-time PCR instruments.


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

<table>
<thead>
<tr>
<th>Component</th>
<th>Label</th>
<th>Contents / Function / Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>food</strong>\textsuperscript{®} Listeria monocytogenes Detection LyoKit</td>
<td>Aluminum bag containing a 8-tube strip mat • R 602 23-1 with white low profile tubes* • R 602 23-2 with clear regular profile tubes* • R 602 23-3 with clear low profile tubes*</td>
<td>- 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and hydrolysis probes specific for <em>Listeria monocytogenes</em> DNA and the Internal Control (IC) as well as Taq DNA Polymerase and Uracl-DNA Glycosylase (UNG, heat labile) for prevention of carry-over contamination. • For amplification and detection of <em>Listeria monocytogenes</em> specific sequences. • Store at 2 °C to 8 °C in the aluminum bag (sealed and containing silica gel pads). • Protect from light and moisture!</td>
</tr>
<tr>
<td>Control Template</td>
<td>Vial 2 (purple cap)</td>
<td>• 1 x 250 µl • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.</td>
</tr>
<tr>
<td>H₂O PCR-grade</td>
<td>Vial 3 (colorless cap)</td>
<td>• 2 x 1 ml • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control.</td>
</tr>
<tr>
<td>Cap strips</td>
<td>Plastic bag containing 8-cap strips</td>
<td>• 12 x 8-cap strip • For use in real-time PCR after addition of samples.</td>
</tr>
</tbody>
</table>

\*Tube profile and instrument compatibility chart is available online: [www.bc-diagnostics.com/compatibility-chart](http://www.bc-diagnostics.com/compatibility-chart)

Additional Equipment and Reagents Required
- Real-time PCR cycler 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 Kits
  - **food**\textsuperscript{®} StarPrep Two Kit (Order No. S 400 08)\textsuperscript{1}
  - **food**\textsuperscript{®} StarPrep Two 8-strip Kit (Order No. S 400 17 L)\textsuperscript{1}
  - **food**\textsuperscript{®} ShortPrep II Kit (Order No. S 400 02)\textsuperscript{1} or
  - **food**\textsuperscript{®} Magnetic Preparation Kit II (Order No. S 400 12)\textsuperscript{1}
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex centrifuge Multispin MSC-6000 for PCR-strips (Order No. D 110 66)\textsuperscript{1} with
- SR-32, Rotor for MSC-3000/6000 (Order No. D 110 65)\textsuperscript{1} or
- Vortex centrifuge CVP-2 for PCR-plates (Order No. D 110 67)\textsuperscript{1}

\textsuperscript{1} Available from BIOTECON Diagnostics; see ordering information for details

Applicability Statement
The **food**\textsuperscript{®} Listeria monocytogenes Detection LyoKit – 5’Nuclease – is intended for the rapid detection of *Listeria monocytogenes* isolated by **food**\textsuperscript{®} DNA extraction methods from enrichment cultures of all relevant kind of foods and primary production stage (PPS) samples that are potentially contaminated with *Listeria monocytogenes*. 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® (Agilent Technologies), ABI 7500 fast (Applied Biosystems), CFX96 (Bio-Rad) and PikoReal® 24 (Thermo Scientific). The performance of the foodproof® Listeria monocytogenes Detection LyoKit in combination with several foodproof® DNA extraction procedures (foodproof® ShortPrep II Kit, foodproof® StarPrep Two procedure A and B, foodproof® StarPrep Two 8-strip and foodproof® Magnetic Preparation Kit II) has been approved in an AOAC-RI PTM method extension (certificate No. 070401). For this validation study the following food categories were tested: raw meat, processed meat, seafood, milk and dairy and fruit/juices. For further information about the tested matrices, enrichment and the DNA extraction procedure please refer to ANNEX 1 at the end of the package insert.

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 monocytogenes DNA using the foodproof® Listeria monocytogenes 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 monocytogenes 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 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 monocytogenes 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.
2.2 Procedure
Program Setup
The following procedure is optimized for a real-time PCR instrument with a FAM (for *Listeria monocytogenes*) and VIC/Yakima Yellow (for Internal Amplification Control) detection channel. Program the PCR instrument before preparing the samples. Use the following real-time PCR-protocol for the foodproof® *Listeria monocytogenes* Detection LyoKit. For details on how to program the experimental protocol, see the Instrument Operator’s Manual of your real-time PCR-cycler:

**Pre-incubation**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37 °C</td>
<td>4 minutes</td>
</tr>
<tr>
<td>2</td>
<td>95 °C</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

**Amplification**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95 °C</td>
<td>5* seconds</td>
</tr>
<tr>
<td>2**</td>
<td>60 °C</td>
<td>60 seconds</td>
</tr>
</tbody>
</table>

* Please use 15 seconds for the LightCycler 96
** Fluorescence detection in step 2

Notes:
- For instruments without VIC, HEX can be used. For the PikoReal® 24, Yakima Yellow (YY) has to be chosen.
- 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® *Listeria monocytogenes* Detection LyoKit contains probes with a non-fluorescent (“dark”) 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 the Filter Set Gain Setting has to be modified to “x1”.

Preparation of the PCR Mix
Proceed as described below to prepare a 25 µl standard reaction. Always wear gloves when handling strips or caps. Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors.

Note: The PCR strips must be stored in the provided aluminum bag with the silica gel pads to avoid liquid absorption.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or scalpel to cut the strips apart. Tightly seal the bag afterwards and store away at the recommended conditions.

2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. Check that the reagent pellets are at the bottom of the tubes. If not, briefly centrifuge or flick the pellets to the bottom before proceeding.

3. Decap the tube strips cautiously and discard the cap strips.

Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips only shortly before filling.

4. Pipet 25 µl sample into each PCR-vessel:
   - For the samples of interest, add 25 µl sample DNA (if using less volume, add PCR-grade H₂O to achieve 25 µl).
   - For the negative control, add 25 µl PCR-grade H₂O (vial 3, colorless cap).
   - For the positive control, add 25 µl foodproof® *Listeria monocytogenes* Detection Control Template (vial 2, purple cap).

Note: To reduce the risk of cross-contamination, it is recommended to prepare only one PCR tube strip at a time.
5. Seal the vessels accurately and tightly with the colorless cap strips.

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 1000 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 monocytogenes*-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 monocytogenes*) and channel VIC/YY (Internal Amplification Control) for each sample, and interpret the results as described in the table below.

<table>
<thead>
<tr>
<th>Channel FAM</th>
<th>Channel VIC/YY</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive or Negative</td>
<td>Positive for <em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Negative for <em>Listeria monocytogenes</em></td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Invalid</td>
</tr>
</tbody>
</table>

**Note:** A prerequisite for the unambiguous discrimination of *Listeria monocytogenes* 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

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Reason</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal increase is observed, even with positive controls.</td>
<td>Incorrect detection channel has been chosen.</td>
<td>• Set Channel settings to FAM or VIC/YY.</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors.</td>
<td>• Check for correct reaction setup. Repeat the PCR run.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Always run a positive control along with your samples.</td>
</tr>
<tr>
<td></td>
<td>No data acquisition programmed.</td>
<td>• Check the cycle programs.</td>
</tr>
<tr>
<td>No signal increase in channel VIC/YY is observed.</td>
<td>Inhibitory effects of the sample material (e.g., caused by insufficient purification).</td>
<td>• Use the recommended DNA sample preparation kit to purify template DNA.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dilute samples or pipet a lower amount of sample DNA (e.g., 5 µl instead of 25 µl).</td>
</tr>
<tr>
<td>Fluorescence intensity is too low.</td>
<td>Inappropriate storage of kit components.</td>
<td>• Store the foodproof® Listeria monocytogenes Detection lyophilized PCR Mix at 2 °C to 8 °C, protected from light and moisture.</td>
</tr>
<tr>
<td></td>
<td>Low initial amount of target DNA.</td>
<td>• Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.</td>
</tr>
<tr>
<td>Strong decrease of fluorescence baseline.</td>
<td>Resuspension of lyophilized PCR mix not complete.</td>
<td>• Always resuspend lyophilized PCR mix thoroughly.</td>
</tr>
<tr>
<td>Negative control samples are positive.</td>
<td>Carry-over contamination.</td>
<td>• Exchange all critical solutions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Repeat the complete experiment with fresh aliquots of all reagents.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Add positive controls after sample and negative control reaction vessels have been sealed.</td>
</tr>
<tr>
<td>Fluorescence intensity varies.</td>
<td>Insufficient centrifugation of the PCR strips. Resuspend PCR mix is still in the upper part of the vessel.</td>
<td>• Always centrifuge PCR strips.</td>
</tr>
<tr>
<td></td>
<td>Outer surface of the vessel or the seal is dirty (e.g., by direct skin contact).</td>
<td>• Always wear gloves when handling the vessels and seal.</td>
</tr>
<tr>
<td>Pellets are difficult to dissolve.</td>
<td>The lyophilized PCR mix started to rehydrate.</td>
<td>• Store the lyophilized PCR mix always in the aluminum bag with the silica gel pad</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Open Strip shortly before filling.</td>
</tr>
</tbody>
</table>

4. Additional Information on this Product

How this Product Works

The foodproof® Listeria monocytogenes 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 monocytogenes-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 monocytogenes in the sample. The foodproof® Listeria monocytogenes Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of Listeria monocytogenes-DNA. Primers and probes provide specific detection of Listeria monocytogenes-DNA in food samples, including PPS. 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 monocytogenes*.
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 monocytogenes* 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 monocytogenes* Detection LyoKit, decontamination can be achieved with the provided reagents.

**Background Information**

The genus *Listeria* includes ten species (gram-positive rod-shaped bacteria), among which only *Listeria monocytogenes* causes severe disease in humans. Manifestations of Listeriosis include meningoencephalitis, septicemia and abortion. The mortality rate is up to 33%. The most vulnerable people are pregnant women and their infants, the elderly, and those who are immunosuppressed. Every year in the U.S. approximately 2,500 cases of Listeriosis are known to occur (It is likely that more cases remain undetected.) About 500 deaths per year are attributed to Listeriosis [1]. Infections with *Listeria monocytogenes* have been traced to the consumption of contaminated foods, mainly dairy products, meat, and raw vegetables [2]. Because most foods investigated have relatively short shelf lives, the need for rapid, accurate, and sensitive methods for the detection of *Listeria monocytogenes* is a major food safety issue. Since conventional microbiological methods for the detection and identification of *Listeria monocytogenes* are very time-consuming, PCR has been introduced to the food industry as a highly sensitive and specific detection method [3].

**References**


**Quality Control**

The foodproof® *Listeria monocytogenes* Detection LyoKit is function tested using the LightCycler® 480 System.
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
Version 1, September 2014
First version of the package insert.
Version 2, March 2017
License Notice changed.
Introduction of vortex centrifuges into the PCR Setup Procedure.
Version 3, December 2019
Introduction of ANNEX 1: AOAC-RI #070401 Extension Table and additional information in the Applicability Statement.
Introduction of R 602 23-3, new tube format for other real-time PCR instruments.
Introduction of AOAC-RI logo.
ANNEX 1: AOAC-RI #070401 Extension Table for the foodproof® *Listeria monocytogenes* Detection LyoKit

The following table shows the recommended enrichment time for different food matrices with enrichment in 1/2 Fraser broth in combination with different foodproof® DNA extraction procedures that have been validated for the AOAC-RI PTM extension of the foodproof® *Listeria monocytogenes* Detection LyoKit.

For further information regarding the DNA extraction procedures below, please refer to the appropriate BIOTECON Diagnostics package inserts on: www.bc-diagnostics.com.

<table>
<thead>
<tr>
<th>DNA Extraction</th>
<th>Enrichment time in Half Fraser broth at 30°C</th>
<th>Enrichment time per matrix</th>
</tr>
</thead>
</table>
| foodproof® ShortPrep II Kit         | 24 h – 48 h                                 | minced meat 48 h  
                                           |                                                             | melon 24 h  
                                           |                                                             | raw fish 48 h  
                                           |                                                             | sausage 48 h  
                                           |                                                             | Harzer cheese 48 h |
| foodproof® StarPrep Two procedure A | 24 h                                        | minced meat 24 h  
                                           |                                                             | melon 24 h  
                                           |                                                             | raw fish 24 h  
                                           |                                                             | sausage 24 h  
                                           |                                                             | Harzer cheese 24 h |
| foodproof® StarPrep Two procedure B | 24 h – 48 h                                 | minced meat 48 h  
                                           |                                                             | melon 24 h  
                                           |                                                             | raw fish 48 h  
                                           |                                                             | sausage 48 h  
                                           |                                                             | Harzer cheese 48 h |
| foodproof® StarPrep Two 8-strip     | 48 h                                        | minced meat 48 h  
                                           |                                                             | melon 48 h  
                                           |                                                             | raw fish 48 h  
                                           |                                                             | sausage 48 h  
                                           |                                                             | Harzer cheese 48 h |
| foodproof® Magnetic Preparation Kit II | 24 h – 48 h                               | minced meat 48 h  
                                           |                                                             | melon 24 h  
                                           |                                                             | raw fish 24 h  
                                           |                                                             | sausage 48 h  
                                           |                                                             | Harzer cheese 48 h |

Tested food categories: raw meats, processed meats, seafood, milk and dairy, fruit/juices

Sample size: 25 g / 225 ml broth