



For food testing purposes.
FOR *IN VITRO* USE ONLY.

foodproof® *Listeria* Genus Detection Kit - Hybridization Probes (LC 1.x, 2.0) -

Version 4, September 2017

PCR kit for the qualitative detection of *Listeria* DNA using the LightCycler® Carousel-Based System

Order No. R 300 20

Kit for 96 reactions for a maximum of 90 samples

Store the kit at -15 to -25 °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 20 µl each. Up to 30 samples (single sample preparation) plus positive and negative control reactions can be analyzed per LightCycler® Carousel-Based System run (i.e., the complete kit allows analysis of a maximum of 90 samples).

Storage and Stability

The kit is shipped on dry ice.

- 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 / Cap Color	Label	Contents / Function / Storage
1 yellow cap	foodproof <i>Listeria</i> Genus Master Mix	<ul style="list-style-type: none"> • 3 x 420 µl • Ready-to-use primer and Hybridization Probe mix specific for <i>Listeria</i> DNA and the <i>Listeria</i>-specific Internal Control (IC). • For amplification and detection of <i>Listeria</i> specific sequences. • Store at -15 to -25°C. • Avoid repeated freezing and thawing! • Protect from light!
2 red cap	foodproof <i>Listeria</i> Genus Enzyme Solution	<ul style="list-style-type: none"> • 3 x 32 µl • Contains FastStart 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 <i>Listeria</i> Genus Internal Control	<ul style="list-style-type: none"> • 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 °C to +8 °C for up to one month.
4 purple cap	foodproof <i>Listeria</i> Genus Control Template	<ul style="list-style-type: none"> • 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 °C to +8 °C for up to one month.
5 colorless cap	H ₂ O, PCR-grade	<ul style="list-style-type: none"> • 1 x 1 ml • Nuclease-free, PCR-grade H₂O. • For use as a PCR run negative control. • Store at -15 to -25°C.



Additional Equipment and Reagents Required

- LightCycler® Carousel-Based System (LightCycler® 1.x, 2.0 Instrument, Roche Applied Science)²
- LightCycler® 20 µl - Capillaries²
- LightCycler® Color Compensation Set²
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.

The LightCycler® Carousel-Based System provides adapters that allow LightCycler® Capillaries to be centrifuged in a standard microcentrifuge rotor.

or

- LC Carousel Centrifuge 2.0² for use with the LightCycler® 2.0 Sample Carousel (optional).

If you use a LightCycler® Instrument version below 2.0 you need in addition the LC Carousel Centrifuge 2.0 Bucket 2.1².

To adapt the LightCycler® 2.0 Sample Carousel to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set².

- **foodproof** ShortPrep II Kit¹

or

- **foodproof** StarPrep Two Kit¹

or

- **foodproof** Sample Preparation Kit II¹

- Nuclease-free, aerosol-resistant pipette tips
- Pipettes with disposable, positive-displacement tips
- Sterile reaction tubes for preparing PCR mixes and dilutions

¹ Available from BIOTECON Diagnostics; see Ordering Information for details

² Available from Roche Diagnostics

Applicability Statement

The **foodproof** *Listeria* Genus Detection Kit is designed as a screening test for the occurrence of the six *Listeria* species in food samples (*L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri*, *L. welshimeri*). The test allows the rapid detection of *Listeria* DNA isolated from enrichment cultures prepared with various methods inoculated with food samples that are potentially contaminated with *Listeria* spp. It must not be used in diagnostic procedures.

Note: The presence of the pathogenic species *Listeria monocytogenes* in positive samples might be further confirmed by using specific detection methods (e.g., **foodproof** *Listeria monocytogenes* Detection Kit, Cat. No. R 300 23).

The kit must not be used in diagnostic procedures.

The kit described in this Instruction Manual has been developed for the LightCycler® Carousel-Based System.

Assay Time

Procedure	Time
PCR-Setup	15 min
LightCycler® Carousel-Based System PCR run	55 min
Total assay time	70 min

2. How to Use this Product

2.1 Before You Begin

Precautions

Detection of *Listeria* DNA using the **foodproof** *Listeria* Genus Detection Kit requires DNA amplification by PCR. The kit provides all the 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:

- 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-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.
- Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.
- 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.
- In order to avoid cross-contamination, close all capillaries that contain sample DNA and negative controls before pipetting positive controls.

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



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.

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).

Enrichment

Pre-enrichment broth and temperature according to ISO 11290 or BAM (Chapter 10) or USDA for 24 – 48 h. Other suitable, validated enrichment procedures can also be used.

DNA-Extraction

BIOTECON Diagnostics provides sample preparation kits suitable for all kind of foods and raw materials (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 Control Template (vial 4, purple cap)] or with a positive sample preparation control. Always close capillaries with template DNA and negative controls before adding positive control DNA.

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H₂O, 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

LightCycler® Carousel-Based System Protocol

The following procedure is optimized for use with the LightCycler® Carousel- Based System. Program the LightCycler® Carousel-Based System before preparing the reaction mixes. A LightCycler® Carousel-Based System protocol that uses the **foodproof** *Listeria* Genus Detection Kit contains the following programs (for details on how to program the experimental protocol, see the LightCycler® Instrument Operator's Manual):

Pre-incubation (prevention of carry-over contamination, activation of FastStart Taq DNA polymerase, denaturation of template DNA)		
Programs/Cycle Program Data	Value	
Cycles	1	
Analysis Mode	None	
Temperature Targets	Segment 1	Segment 2
Target/Target Temperature [°C]	40	95
Hold/Incubation Time [h:min:s]	00:02:00	00:10:00
Ramp Rate/Temperature Transition Rate [°C/s]	20	20
Sec Target/Secondary Target Temperature [°C]	0	0
Step Size [°C]	0.0	0.0
Step Delay [cycles]	0	0
Acquisition Mode	None	None

Amplification (of the target DNA)			
Programs/Cycle Program Data		Value	
Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target/Target Temperature [°C]	95	59	72
Hold/Incubation Time [h:min:s]	00:00:00	00:00:30	00:00:05
Ramp Rate/Temperature Transition Rate [°C/s]	20	20	20
Sec Target/Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [cycles]	0	0	0
Acquisition Mode	None	Single	None
Cooling (of the rotor and thermal chamber)			
Programs/Cycle Program Data		Value	
Cycles	1		
Analysis Mode	None		
Temperature Targets	Segment 1		
Target/Target Temperature [°C]	40		
Hold/Incubation Time [h:min:s]	00:00:30		
Ramp Rate/Temperature Transition Rate [°C/s]	20		
Sec Target/Secondary Target Temperature [°C]	0		
Step Size [°C]	0.0		
Step Delay [cycles]	0		
Acquisition Mode	None		

Fluorescence and Run Setup Parameters

Parameter	Setting	
All LightCycler® Software Versions		
Seek Temperature	30°C	
LightCycler® Software prior to Version 3.5		
Display Mode	Fluorescence channel F2 or F3	
Fluorescence Gains	Fluorimeter	Gain Value
	Channel 1 (F1)	1
	Channel 2 (F2)	15
	Channel 3 (F3)	30
LightCycler® Software Version 3.5		
Display Mode • during run • for analysis	<ul style="list-style-type: none"> Fluorescence channel F2 or F3 F2/Back-F1 or F3/Back-F1 	
Fluorescence Gains	not required In data created with LightCycler® Software Version 3.5, all fluorescence values are normalized to a fluorescence gain of "1". This produces a different scale on the Y-axis than that obtained with previous LightCycler® Software Versions. This difference does not affect the crossing points nor any calculated concentrations obtained.	
LightCycler® Software Version 4.x		
Default channel • during run • for analysis	<ul style="list-style-type: none"> Fluorescence channel 640 or 705 640/Back 530 or 705/Back 530 	
Fluorescence Gains	not required	
"Max. Seek Pos"	Enter the number of samples including controls.	
"Instrument Type"	"6 Ch.": for LightCycler® 2.0 Instrument (selected by default). "3 Ch.": for LightCycler® 1.5 Instrument and instrument versions below.	
"Capillary Size"	Select "20 µl" as the capillary size for the experiment (for the "6 Ch." instrument type only).	

Preparation of the PCR Mix

Proceed as described below to prepare a 20 µl standard reaction. Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.

1. Depending on the total number of reactions, place the required number of LightCycler® Capillaries in centrifuge adapters or in a LightCycler® Sample Carousel in a LC Carousel Centrifuge Bucket.
2. 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.
3. In a 1.5 ml reaction tube, prepare the PCR Mix by adding the following components in the order mentioned below, then mix gently but thoroughly by pipetting up and down:

The volumes indicated below are based on a single 20 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 <i>Listeria</i> Genus Master Mix, (vial 1, yellow cap)	13 µl
foodproof <i>Listeria</i> Genus Enzyme Solution, (vial 2, red cap)	1 µl
foodproof <i>Listeria</i> Genus Internal Control, (vial 3, white cap)	1 µl
Total volume	15 µl

4. • Mix carefully but thoroughly by pipetting up and down. Do not vortex.
 - Pipet 15 µl PCR mix into each LightCycler® capillary.
 - For the samples of interest, add 5 µl sample DNA to a capillary, seal with a stopper.
 - For the negative control, add 5 µl H₂O, PCR-grade (vial 5, colorless cap), seal with a stopper.
 - For the positive control, add 5 µl foodproof *Listeria* Genus Control Template (vial 4, purple cap), seal with a stopper.
5. • Place the adapters (containing the capillaries) in a standard benchtop microcentrifuge. (Place the centrifuge adapters in a balanced arrangement within the centrifuge.)
 - Centrifuge at 700 x g for 5 s (3,000 rpm in a standard benchtop microcentrifuge).
 - Alternatively, use the LC Carousel Centrifuge for spinning the capillaries.
6. Transfer the capillaries to the LightCycler®.
7. Cycle the samples as described above.



2.3 Analysis

Color Compensation

The use of the previously generated color compensation file or color compensation object is a prerequisite for the unambiguous discrimination of *Listeria* DNA and internal control (IC) DNA amplification in this dual-color experiment. For additional information on the generation and use of a color compensation file or object, refer to the LightCycler® Instrument Operator's Manual and to the pack insert of the LightCycler® Color Compensation Set.

Users of **LightCycler® Software 3.5** proceed as described below to use a stored color compensation file **after the PCR** run on the LightCycler® Carousel-Based System:

1. Select the data file in the LightCycler® Data Analysis module of the LightCycler® Software.
2. Click on the Select a Program button and select the program to be analyzed.
3. Under the Color Compensation menu, select Load Calibration Data, then highlight the stored 'CCC' color compensation file. Alternatively, click on the Select CCC Data button and choose Import CCC File.
4. To display the color compensated data, click on the Color Compensation button. Alternatively, select Enable under the Color Compensation pull-down menu.
5. To return to the raw data, click on the Color Compensation button again. Alternatively, select Disable under Color Compensation pull-down menu.

Users of **LightCycler® Software 4.x** proceed as described below to use a stored color compensation object after the PCR run on the LightCycler® Carousel-Based System:

1. Add the analysis module, click Color Compensation in the analysis window, then select Select Color Compensation... .
2. Select the color compensation object you want to apply, then click OK.
3. A small dialog box opens so you can select the channels to compensate. The number of channels displayed depends on the number of channels used in the color compensation experiment. By default all channels are selected.
4. Deselect any channels you do not want to compensate (*i.e.*, for this kit select channels 530, 640, and 705 only), then click OK.
5. The analysis charts are redrawn using the compensated data. Notice that the Color Compensation menu label now says "(On)".



Data Interpretation

Analyze real-time PCR results in channels F2/Back-F1 and F3/Back-F1 (LightCycler® Software 3.5 and software versions below) or in channels 640/Back 530 and 705/Back 530 (LightCycler® Software 4.x) respectively, using the Qualitative Detection module of the LightCycler® Analysis Software. Check for a positive result of the Internal Control (visible signal in channel F3 or 705) for each sample that is negative for *Listeria* DNA (no signal in channel F2 or 640). Compare the results from channel F2 or 640 (*Listeria*) and channel F3 or 705 (Internal Control) for each sample, and interpret the results as described in the table below:

<i>Listeria</i> spp. Channel F2/Back-F1 or Channel 640/Back 530	Internal Control Channel F3/Back-F1 or Channel 705/Back 530	Result Interpretation
Positive	Positive	Positive
Negative	Positive	Negative
Positive	Negative	Positive
Negative	Negative	Invalid

Note:

Always verify software results (red signals for positive samples/green signals for negative samples) for plausibility by inspection of the amplification curves.

3. Troubleshooting

Observations	Possible Cause	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	Set Channel settings to F2 (640) or F3 (705). Fluorescence data is acquired for all channels during the run, regardless of the channel settings. If the incorrect channel is selected, there is NO need to abort and redo the run.
	Pipetting errors or omitted reagents.	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • Check the cycle programs. • Select acquisition mode "single" at the end of each annealing segment of the PCR program.
No signal increase in channel F3/Back-F1 (705/Back 530) 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., 2.5 µl instead of 5 µl). • Perform a sub-cultivation of the enrichment culture (e.g., 1:100 in broth according to Fraser) to dilute the portion of food matrix in the sample.
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> • Store the foodproof Listeria Genus Master Mix (vial 1, yellow cap) at -15 °C to -25 °C, protected from light. • Avoid repeated freezing and thawing.
	foodproof Listeria Genus Master Mix (vial 1, yellow cap) is not homogeneously mixed.	Mix the foodproof Listeria Genus 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.	<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 capillaries and negative control capillaries have been sealed with stoppers.
Fluorescence intensity varies.	Insufficient centrifugation of the capillaries. Prepared PCR mix is still in the upper vessel of the capillary. Air bubble is trapped in the capillary tip.	Always centrifuge capillaries (loaded with the reaction mix) as described.
	Outer surface of the capillary tip is dirty (e.g., by direct skin contact).	Always wear gloves when handling the capillaries.



4. Additional Information on this Product

How this Product Works

The **foodproof** *Listeria* Genus Detection Kit provides primers and Hybridization Probes (for sequence-specific detection), convenient premixed 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 supplied with the kit (vial 3, white cap). The IC has to be added to each reaction. Hybridization Probes were designed to bind specifically the IC, allowing detection in channel F3 (LightCycler® Software 3.5 and versions below) or 705 (LightCycler® Software 4.x), whereas the *Listeria* Genus DNA is detected in channel F2 (LightCycler® Software 3.5 and versions below) or 640 (LightCycler® Software 4.x). 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. Whereas a negative result for the sample DNA of interest and amplification of the IC clearly indicates the absence of *Listeria* DNA in the sample. The **foodproof** *Listeria* Genus Detection Kit minimizes contamination risk and contains all reagents (except for template DNA) needed for detection of *Listeria* DNA. The Kit is specifically adapted for PCR in glass capillaries using the LightCycler® Carousel-Based System. Primers and Hybridization Probe probes provide specific detection of *Listeria* DNA in food samples. The kit described in this Manual Instruction has been developed for the LightCycler® Carousel-Based System.

Test Principle

1. Using the kit's supplied sequence-specific primers in a polymerase chain reaction (PCR), the LightCycler® Carousel-Based System and its associated reagents amplify and simultaneously detect fragments of a *Listeria* specific sequence.
2. The LightCycler® Carousel-Based System detects these amplified fragments in real time through fluorescence generated by their corresponding pair of sequence-specific Hybridization Probes. For each amplicon, one probe is labeled at the 5'-end with an acceptor fluorophore and, to avoid extension, is modified at the 3'-end by phosphorylation. The other oligonucleotide probe is labeled at the 3'-end with a donor fluorophore.
3. During the annealing phase of each PCR cycle, these probes hybridize to an internal sequence of the amplicon. Only while hybridized in close proximity to each other do these probes result in fluorescence resonance energy transfer (FRET) between the two fluorophores. During FRET, the light source of the LightCycler® Carousel-Based System excites the donor fluorophore and part of the excitation energy is transferred to the acceptor fluorophore.
4. The LightCycler® Instrument measures the emitted fluorescence of the acceptor fluorophore.



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

Background Information

The genus *Listeria* includes six species, *L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*, characterized as gram-positive rod-shaped bacteria. Only *L. monocytogenes* causes Listeriosis, a severe human disease. Main clinical manifestations of Listeriosis are meningo-encephalitis and septicemia. The mortality rate is up to 33% [1]. Most susceptible persons are pregnant women, neonates, the elderly and immunosuppressed persons. *L. monocytogenes* is often found in samples that contain other *Listeria* spp. Therefore, the survey for all *Listeria* spp. might be a useful indicator of sanitation and an early warning of potential presence of pathogenic species [2].

The bacteria *Listeria* is typically transmitted to humans through the ingestion of contaminated food such as dairy products, meat and raw vegetables [3]. Those foods have relatively short shelf lives, and food industries have to shorten in-process control to prevent in-house contamination. Therefore the need for rapid, accurate, and sensitive methods for the detection of *Listeria* is a major food safety issue. Since conventional microbiological methods for the detection and identification of *Listeria* are very time-consuming, PCR has been introduced to the food industry as a highly sensitive and specific detection method [4].

Product characteristics

Specificity: The **foodproof *Listeria* Genus Master Mix** is sequence-specific for all *Listeria* species. Inclusivity was tested using 105 strains of all 6 species (*L. grayi*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. seeligeri*, and *L. welshimeri*). Exclusivity was determined by using 56 non-*Listeria* strains.

Sensitivity: A relative detection limit of 1 to 10 cells per 25 g sample can be achieved with all kinds of foods. The **foodproof *Listeria* Genus Detection kit** detects down to 10^2 - 10^3 cfu/ml in enrichment cultures (depending on the sample preparation kit used: **foodproof ShortPrep II Kit** or **foodproof Sample Preparation Kit II**, respectively).

References

1. Centers for Disease Control and Prevention – Listeriosis <http://www.cdc.gov>.
2. Robinson R.K., Batt C.A., Patel P.D. (ed.) 2000. Encyclopedia of food microbiology. volume 2, Academic Press.
3. Scheu P., Gasch A, Berghof K. 1999. Rapid detection of *Listeria monocytogenes* by PCR-ELISA. *Letters in Applied Microbiology* **29**, 416-420.
4. Scheu P.M., Berghof K, Stahl U. 1998. Detection of pathogenic and spoilage micro-organisms in food with the polymerase chain reaction. *Food Microbiology* **15**, 13-31.
5. Fraser, J.A. und Sperber, W.H. 1988. *J. Food Protect.* **51**, Nr. 10, 762-765

Quality Control

The **foodproof *Listeria* Genus Detection Kit** is function tested using the LightCycler® Carousel-Based 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.

LIGHTCYCLER and HYBPROBE are trademarks of Roche.

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 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 2, April 2016

Version 3, March 2017

License Notice changed.

Version 4, September 2017

License Notice changed.

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