



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

## **foodproof® *Escherichia coli* and *Shigella* Detection Kit** **- Hybridization Probes (LC 1.x, 2.0, 480 II) -**

**Version 3, September 2017**

PCR kit for the qualitative detection of *Escherichia coli* and *Shigella* spp.  
DNA using the LightCycler® Carousel-Based or 480 II System

**Order No. R 300 09**

Kit for 96 reactions

**Store the kit at -15 to -25°C**

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**1. What this Product Does**

**Number of Tests**

The detection 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 detection kit allows analysis of a maximum of 90 samples). Using the LightCycler® 480 System up to 94 samples can be analyzed.

**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 kit components as described in the following Kit Contents table:

**Kit Contents**

Vial / Cap Color	Label	Contents / Function / Storage
1 yellow cap	foodproof <i>E. coli</i> and <i>Shigella</i> Master Mix	<ul style="list-style-type: none"> <li>• 3 x 420 µl</li> <li>• Ready-to-use primer and Hybridization Probe mix specific for <i>E. coli</i> and <i>Shigella</i> DNA and <i>E. coli</i> and <i>Shigella</i>-specific Internal Control (IC).</li> <li>• For amplification and detection of <i>E. coli</i> and <i>Shigella</i> specific sequences</li> <li>• Store at -15 °C to -25 °C.</li> <li>• Avoid repeated freezing and thawing!</li> <li>• Protect from light!</li> </ul>
2 red cap	foodproof <i>E. coli</i> and <i>Shigella</i> Enzyme Solution	<ul style="list-style-type: none"> <li>• 3 x 32 µl</li> <li>• Contains DNA-free Taq DNA Polymerase and Uracil-DNA Glycosylase (heat labile) for prevention of carry-over contamination.</li> <li>• Store at -15 °C to -25 °C</li> </ul>
3 white cap	foodproof <i>E. coli</i> and <i>Shigella</i> Internal Control	<ul style="list-style-type: none"> <li>• 3 x 32 µl</li> <li>• Contains a stabilized solution of plasmid DNA.</li> <li>• For use as an internal amplification control.</li> <li>• Store at -15 to -25°C.</li> <li>• After first thawing store at +2 °C to +8 °C for up to one month.</li> </ul>
4 purple cap	foodproof <i>E. coli</i> and <i>Shigella</i> Control Template	<ul style="list-style-type: none"> <li>• 1 x 50 µl</li> <li>• Contains a stabilized solution of plasmid DNA.</li> <li>• For use as a PCR run positive control.</li> <li>• Store at -15 to -25°C.</li> <li>• After first thawing store at +2 °C to +8 °C for up to one month.</li> </ul>
5 colorless cap	H <sub>2</sub> O, PCR-grade	<ul style="list-style-type: none"> <li>• 1 x 1 ml</li> <li>• Nuclease-free, PCR-grade H<sub>2</sub>O.</li> <li>• For use as a PCR run negative control.</li> <li>• Store at -15 to -25°C.</li> </ul>



#### Additional Equipment and Reagents Required

- LightCycler® Carousel-Based System (LightCycler® 1.x, 2.0 Instrument)<sup>2</sup>
- LightCycler® 20 µl - Capillaries\*
- 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 2.0<sup>2</sup> 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 2.1<sup>2</sup>. To adapt the LightCycler® 2.0 Sample Carousel to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set<sup>2</sup>.

or

- LightCycler® 480 II System<sup>2</sup>
- LightCycler® 480 II compatible PCR plate and sealing foil<sup>2</sup>

- LightCycler® Color Compensation Set (Cat. No. 12158850001)<sup>2</sup>

- **foodproof** StarPrep One Kit<sup>1</sup> (Cat. No. S 400 07)

or

- **foodproof** ShortPrep II Kit I<sup>1</sup> (Cat. No. S 400 02)

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

<sup>1</sup> Available from BIOTECON Diagnostics; see Ordering Information for details

<sup>2</sup> Available from Roche Diagnostics

#### Applicability Statement

The **foodproof** *Escherichia coli* and *Shigella* Detection Kit is intended for the rapid detection of *E. coli* and *Shigella* DNA isolated from enrichment cultures prepared by various valid methods and inoculated with a variety of foods that are potentially contaminated with *E. coli* or *Shigella* spp.

#### The kit must not be used in diagnostic procedures.

The user should take care with enrichment cultures as they may contain high numbers of pathogenic organisms. It is strictly recommended to follow all necessary precautions for handling pathogenic organisms during the whole analysis.

The kit described in this Instruction Manual has been developed for the LightCycler® Carousel-Based System and the LightCycler® 480 System II (96-well block type). The LightCycler 480 I System should not be used for this kit.



## 2. How to Use this Product

### 2.1 Before You Begin

#### Precautions

Detection of *E. coli* and *Shigella* DNA using the **foodproof** *Escherichia coli* and *Shigella* 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-set-up, and PCR to minimize risk of carry-over contamination.
- In order to avoid cross-contamination, close all capillaries that contain sample DNA and negative controls before pipetting positive controls.

**Keep the foodproof *Escherichia coli* and *Shigella* 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).

#### 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](http://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 *E. coli* and *Shigella* Control Template (vial 4, purple cap)] or with a positive sample preparation control DNA. 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 H<sub>2</sub>O, PCR-grade water (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.

#### **Color Compensation**

The use of a previously generated color compensation object is a prerequisite for the unambiguous discrimination of *E. coli/Shigella* DNA and internal control (IC) DNA amplification in this dual-color experiment. A suitable color compensation object can be generated using dedicated reagents available as LightCycler® Color Compensation Set (Cat. No. 12158850001, Roche Diagnostics). As color compensation is instrument-specific, it is necessary to generate a CC object for every LightCycler® Instrument. A new object has to be created after the optical system has been repaired. For additional information on color compensation please refer to the manual of the respective LightCycler® Instrument.

#### **2.2 Procedure**

The following procedures are optimized for the LightCycler® 480 II System and the LightCycler® 2.0 Carousel-Based System. Program the LightCycler® Systems before preparing the reaction mixes. The protocols contain the following programs:

- Pre-Incubation to prevent carry-over contamination (UNG), to activate Taq polymerase and for DNA-denaturation
- Amplification of the target DNA
- Cooling of the LightCycler® System

**LightCycler® 480 II System Protocol**

The following procedure is optimized for use with the LightCycler® 480 II System. Program the LightCycler® before preparing the reaction mixes. Use the following LightCycler® 480 System PCR-program for the **foodproof** *Escherichia coli* and *Shigella* Detection Kit (for details on how to program the experimental protocol, see the LightCycler® 480 II System Operator's Manual):

Set-Up							
Detection Format		Block Type			Reaction Volume		
Multi Color HybProbe		96			20 µl		
Filter Setting		dynamic mode, LC480 II : Fluos (465-510, Red 640 (498-640) and Cy 5/ Cy 5.5 (498-660)					
Programs							
Program Name		Cycles			Analysis Mode		
Pre-Incubation		1			None		
Amplification		45			Quantification		
Cooling		1			None		
Temperature Targets							
	Target (°C)	Aquisition Mode	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Secondary Target Temperature [°C]	Step Size [°C]	Step Delay [cycles]
Pre-Incubation							
Segment 1	37	None	00:02:00	4.4	0	0.0	0
Segment 2	95	None	00:10:00	4.4	0	0.0	0
Amplification							
Segment 1	95	None	00:00:05	4.4	0	0.0	0
Segment 2	59	Single	00:00:35	2.2	0	0.0	0
Segment 3	72	None	00:00:15	4.4	0	0.0	0
Cooling							
	40	None	00:00:30	2.2	0	0.0	0



**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** *Escherichia coli* and *Shigella* Detection Kit contains the following programs (for details on how to program the experimental protocol, see the LightCycler® Instrument Operator's Manual):

Pre-incubation		
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

<b>Amplification</b>			
<i>Programs/Cycle Program Data</i>	<i>Value</i>		
Cycles	45		
Analysis Mode	Quantification		
<i>Temperature Targets</i>	<i>Segment 1</i>	<i>Segment 2</i>	<i>Segment 3</i>
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
<b>Cooling</b>			
<i>Programs/Cycle Program Data</i>	<i>Value</i>		
Cycles	1		
Analysis Mode	None		
<i>Temperature Targets</i>	<i>Segment 1</i>		
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	
<b>All LightCycler® Software Versions</b>		
Seek Temperature	30°C	
<b>LightCycler® Software prior to Version 3.5</b>		
Display Mode	Fluorescence channel F2 or F3	
Fluorescence Gains	<b>Fluorimeter</b>	<b>Gain Value</b>
	Channel 1 (F1)	1
	Channel 2 (F2)	15
	Channel 3 (F3)	30
<b>LightCycler® Software Version 3.5</b>		
Display Mode • during run • for analysis	<ul style="list-style-type: none"> <li>• Fluorescence channel F2 or F3</li> <li>• F2/Back-F1 or F3/Back-F1</li> </ul>	
Fluorescence Gains	<p>not required</p> <p>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.</p>	
<b>LightCycler® Software Version 4.x</b>		
Default channel • during run • for analysis	<ul style="list-style-type: none"> <li>• Fluorescence channel 640 or 705</li> <li>• 640/Back 530 or 705/Back 530</li> </ul>	
Fluorescence Gains	not required	
"Max. Seek Pos"	Enter the number of samples including controls.	
"Instrument Type"	<p>"6 Ch.": for LightCycler® 2.0 Instrument (selected by default)</p> <p>"3 Ch.": for LightCycler® 1.5 Instrument and instrument versions below</p>	
"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. For LightCycler® 480 users, do not touch the upper surface of the PCR multiwell plate.

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. For LightCycler 480 instruments, use a suitable multiwell plate.
2. Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. Mix carefully by pipetting up and down.
3. In a 1.5 ml reaction tube, prepare the PCR Mix for one 20 µl reaction by adding the following components in the order mentioned below, then mix gently 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 plus one positive and on negative control to be cycled plus one or two additional reactions to cover pipetting losses.

Component	Volume
foodproof <i>E. coli</i> and <i>Shigella</i> Master Mix, (vial 1, yellow cap)	13 µl
foodproof <i>E. coli</i> and <i>Shigella</i> Enzyme Solution (vial 2, red cap)	1 µl
foodproof <i>E. coli</i> and <i>Shigella</i> Internal Control (vial 3, white cap)	1 µl
<b>Total volume</b>	15 µl

4. • Mix carefully by pipetting up and down. Do not vortex.
  - Pipet 15 µl PCR mix into each LightCycler® capillary or plate well respectively.
  - For the samples of interest, add 5 µl sample DNA to a capillary or a well (LC 480), seal the capillary with a stopper.
  - For the negative control, add 5 µl H<sub>2</sub>O, PCR-grade (vial 5, colorless cap), seal the capillary with a stopper.
  - For the positive control, add 5 µl foodproof *E. coli* and *Shigella* Control Template (vial 4, purple cap), seal the capillary with a stopper.
5. • **For LightCycler® Carousel Based System:** 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. Transfer the capillaries to the LightCycler®.
6. **For LightCycler® 480 II System:** Seal the plate accurately with a optical sealing foil. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s.
7. Cycle the samples as described above.



**2.3 Analysis**

Analyze real-time PCR results in channels F2/Back-F1 and F3/Back-F1 (LightCycler® Software 3.5 and software versions below), in channels 640/Back 530 and 705/Back 530 (LightCycler® Software 4.x) or channels Red 640 (498-640) and Cy 5/ Cy 5.5 (498-660) (LightCycler® 480 Software 1.5) respectively, using the Quantification module (LightCycler® Software 3.5 and software versions below), the Qualitative Detection module (LightCycler® Software 4.x) or the Abs Quant/2nd Derivative Max analysis type (LightCycler® 480 Software 1.5) of the LightCycler® Analysis Software. Check for a positive result of the Internal Control (visible signal in channel F3, 705 or Cy5 / Cy 5.5 (498-660)) for each sample that is negative for *E. coli* and *Shigella* DNA (no signal in channel F2, 640 or Red 640 (498-640)). Compare the results from channel F2, 640 or Red 640 (498-640) (*E. coli* and *Shigella*) and channel F3, 705 or Cy 5 / Cy 5.5 (498-660) (Internal Control) for each sample, and interpret the results as described in the table below:

<b><i>E. coli</i> and <i>Shigella</i></b> Channel F2/Back-F1, Channel 640/Back 530 or Red 640 (498-640)	<b>Internal Control</b> Channel F3/Back-F1, Channel 705/Back 530 or Cy 5 / Cy 5.5 (498-660)	<b>Result Interpretation</b>
Positive	Positive	Positive
Negative	Positive	Negative
Positive	Negative	Positive
Negative	Negative	Invalid

**Notes:**

For LightCycler® 480 II System: Use the "High Sensitivity" setting of the LightCycler® Software to calculate results.  
 For LightCycler® 2.0 and LightCycler® 480 II System: Always verify the software results ("positive", "negative", "uncertain") for plausibility by inspection of the amplification curves.

### 3. Troubleshooting

	Possible Cause	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	Set Channel Settings to F2/Back-F1 [640/Back 530, Red 640 (498-640)] or F3/Back-F1 [705/Back 530, Cy 5 / Cy 5.5 (498-660)]. For the carousel-based systems 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"> <li>Check for correct pipetting scheme and reaction setup. Repeat the PCR run.</li> <li>Always run a positive control along with your samples.</li> </ul>
	No data acquisition programmed.	<ul style="list-style-type: none"> <li>Check the cycle programs.</li> <li>Select acquisition mode "single" at the end of each annealing segment of the PCR program.</li> </ul>
No signal increase in channel F3/Back-F1 [705/Back 530, Cy 5 / Cy 5.5 (498-660)] is observed.	Inhibitory effects of the sample material (e.g., caused by insufficient purification).	<ul style="list-style-type: none"> <li>Use the recommended DNA sample preparation kit to purify template DNA.</li> <li>Dilute samples or pipet a lower amount of sample DNA (e.g., 2.5 µl instead of 5 µl).</li> <li>Perform a sub-cultivation of the enrichment culture (e.g., 1:10 in BPW) to dilute the portion of food matrix in the sample.</li> </ul>
Fluorescence intensity is too low.	Inappropriate storage of kit components.	<ul style="list-style-type: none"> <li>Store the <b>foodproof E. coli</b> and <b>Shigella</b> Master Mix (vial 1, yellow cap) at -15 °C to - 25°C, protected from light.</li> <li>Avoid repeated freezing and thawing.</li> </ul>
	<b>foodproof E. coli</b> and <b>Shigella</b> Master Mix (vial 1, yellow cap) is not homogeneously mixed.	Mix the <b>foodproof E. coli</b> and <b>Shigella</b> 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"> <li>Exchange all critical solutions.</li> <li>Repeat the complete experiment with fresh aliquots of all reagents.</li> <li>Always handle samples, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination.</li> <li>Add positive controls after sample capillaries and negative control capillaries have been sealed with stoppers.</li> </ul>
Fluorescence intensity varies.	Insufficient centrifugation of the capillaries or PCR plate.	Always centrifuge capillaries or PCR plate (loaded with the PCR mix) as described.
	Outer surface of the capillary tip or the sealing foil is dirty (e.g., by direct skin contact).	Always wear gloves when handling the capillaries or the sealing foil.



#### 4. Additional Information on this Product

##### How this Product Works

The **foodproof *Escherichia coli* and *Shigella* 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), 705 (LightCycler® Software 4.x) or Cy 5 / Cy 5.5 (498-660) (LightCycler® 480 Software 1.5), whereas the *E. coli* and *Shigella* DNA is detected in channel F2 (LightCycler® Software 3.5 and versions below), 640 (LightCycler® Software 4.x) or Red 640 (498-640) (LightCycler® 480 Software 1.5). In the 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 *E. coli* and *Shigella* DNA in the sample. The **foodproof *Escherichia coli* and *Shigella* Detection Kit** minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of *E. coli* and *Shigella* DNA. The kit is specifically adapted for PCR using the LightCycler® System. Primers and Hybridization Probes provide specific detection of *E. coli* and *Shigella* DNA in food preparations. The kit described in this Instruction Manual has been developed for the LightCycler® 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 detect fragments of *E. coli* and *Shigella* genomic DNA.
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 *E. coli* and *Shigella* 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** *Escherichia coli* and *Shigella* Detection Kit, decontamination can be achieved with the provided reagents.

#### **Quality Control**

The **foodproof** *Escherichia coli* and *Shigella* 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](http://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](mailto: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](http://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, August 2009*

First version of the package insert.

*Version 2; March 2017*

License Notice changed.

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