foodproof® *E. coli* and *Shigella* Detection Kit
- Hybridization Probes (LC 1.x, 2.0, 480 II) - Order No. R 300 09

Quick Reference Procedure (for LC 480 II)
Version 2, March 2012

A. LightCycler® 480 II System Protocol

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

Set-Up		
Detection Format	**Block Type**	**Reaction Volume**
Multi Color HybProbe	96	20 µl
Filter Combination	dynamic mode, 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)	**Acquisition Mode**	**Hold (hh:mm:ss)**	**Ramp Rate (°C/s)**	**Acquisitions (per °C)**
Pre-Incubation					
Segment 1	37	None	00:02:00	4.4	
Segment 2	95	None	00:10:00	4.4	
Amplification					
Segment 1	95	None	00:00:05	4.4	
Segment 2	59	Single	00:00:35	2.2	
Segment 3	72	None	00:00:15	4.4	
Cooling					
Segment 1	40	None	00:00:30	2.2	

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B. Preparation of the PCR Mix

Proceed as described below to prepare a 20 µl standard reaction.

Note: The kit contains one internal amplification control which is used for both types of instruments (LightCycler 2.0 and LightCycler 480 II), the **food**proof *E. coli* and *Shigella* Internal Control (vial 3, white cap).

Always wear gloves when handling the PCR multi-well plates. Do not touch the upper surface of the plates.

1. Use a suitable PCR multi-well plate for LightCycler 480 instruments II.
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:

Component	Volume
foodproof *E. coli* and *Shigella* Master Mix, (vial 1, yellow cap)	13 µl
foodproof *E. coli* and *Shigella* Enzyme Solution, (vial 2, red cap)	1 µl
foodproof *E. coli* and *Shigella* Internal Control (LC 480), (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 well of the PCR plate.
   - For the samples of interest, add 5 µl sample DNA to a well.
   - For the negative control, add 5 µl H_2O, PCR-grade (vial 5, colorless cap) to the well.
   - For the positive control, add 5 µl **food**proof *E. coli* and *Shigella* Control Template (vial 4, purple cap) to a well.
   - Seal the plate accurately with an optical sealing foil.
5. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s.
6. Transfer the PCR plate to the LightCycler® 480 Instrument II.
7. Cycle the samples as described above.

***Note:** For further information please refer to: www.bc-diagnostics.com/?cid=1254812067&lang=1

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