

**foodproof® *E. coli* and *Shigella* Detection Kit**  
**- Hybridization Probes (LC 1.x, 2.0) -**

**Catalog No. R 300 09**

**Quick Reference Procedure**

**A. 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 *E. 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 (prevention of carry-over contamination, activation of 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

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Cooling (of 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

#### 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.

- 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.
- 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.
- 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>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

- 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 H<sub>2</sub>O, PCR-grade (vial 5, colorless cap), seal with a stopper.
  - For the positive control, add 5 µl foodproof *E. coli* and *Shigella* Control Template (vial 4, purple cap), seal with a stopper.
- 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®.
- Cycle the samples as described above.