foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit - Hybridization Probes (LC 2.0, 480) -

Order No. R 310 15.1
Order No. R 310 15.1 L

Quick Reference Procedure (for LC 480)

Version 2, April 2016

A. LightCycler® 480 System Protocol

The following procedure is optimized for use with the LightCycler® 480 System. Program the LightCycler® 480 System before preparing the reaction mixes. Use the following LightCycler® 480 System PCR-program for the **food**proof® *Enterobacteriaceae* plus *Cronobacter* 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 Setting	dynamic mode, LC 480 I: Fluos (483-533), Red 610 (483-610), Red 640 (483-640) and Cy 5 (483-670) LC480 II : Fluos (465-510, Red 610 (498-610), Red 640 (498-640) and Cy 5/ Cy 5.5 (498-660)	
Programs		
Program Name	**Cycles**	**Analysis Mode**
Pre-Incubation	1	None
Amplification	38	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	00:04:00	4.4	0	0.0	0
Segment 2	95	None	00:05:00	4.4	0	0.0	0
Amplification							
Segment 1	95	None	00:00:10	4.4	0	0.0	0
Segment 2	65	Single	00:00:40	2.2	61	0.2	8
Segment 3	72	None	00:00:25	4.4	0	0.0	0
Cooling							
	40	None	00:00:30	2.2	0	0.0	0

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B. Preparation of the PCR Mix
Proceed as described below to prepare a 20 µl standard reaction.
Do not touch the surface of the PCR plate. Always wear gloves when handling the plates.

1. 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.
2. 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 (sample and control reactions) to be cycled plus one or two additional reactions to cover pipetting losses.

Component	Volume
foodproof *Enterobacteriaceae* plus *Cronobacter* Master Mix, (vial 1, yellow cap)	13 µl
foodproof *Enterobacteriaceae* plus *Cronobacter* Enzyme Solution, (vial 2, red cap)	1 µl
foodproof *Enterobacteriaceae* plus *Cronobacter* Internal Control - LC 480, (vial 6, black cap)	1 µl
Total volume	15 µl

3. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
   - Pipet 15 µl PCR mix into each well.
   - For the samples of interest, add 5 µl sample DNA (if less than 5 µl add H_2O to 5 µl) to a well.
   - For the negative control, add 5 µl H_2O, PCR-grade (vial 5, colorless cap).
   - For the positive control, add 5 µl **food**proof *Enterobacteriaceae* plus *Cronobacter* Control Template (vial 4, purple cap).
4. 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. Cycle the samples as described above.

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