For food testing purposes
FOR *IN VITRO* USE ONLY

foodproof® *Enterobacteriaceae* plus *Cronobacter* Detection Kit

- Hybridization Probes (LC 2.0, 480) -

Version 4, September 2017

PCR kit for the qualitative detection of *Enterobacteriaceae* DNA including the simultaneous identification of *Cronobacter* spp. using the LightCycler® 2.0 or 480 System

Order No. R 310 15.1

PCR kit for 96 reactions

Order No. R 310 15.1 L

PCR kit for 480 reactions

Store at -15 to -25 °C

MICROVAL
European validation and certification organisation

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1. What this Product Does

Number of Tests

The detection kit is designed for 96 reactions or 480 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 or 470 samples can be analyzed.

Contents

Vial / Cap Color	Label	Contents / Function / Storage
1 yellow cap	**food**proof *Enterobacteriaceae* plus *Cronobacter* Master Mix	• R 310 15.1: 3 x 420 µl • R 310 15.1 L: 5 x 1.260 µl • Ready-to-use primer and Hybridization Probe mix specific for *Enterobacteriaceae* or *Cronobacter* DNA respectively as well as the specific Internal Control (IC). • For amplification and detection of *Enterobacteriaceae* and *Cronobacter* specific sequences. • Store at -15 to – 25 °C. • **Avoid repeated freezing and thawing!** • **Protect from light!**
2 red cap	**food**proof *Enterobacteriaceae* plus *Cronobacter* Enzyme Solution	• R 310 15.1: 3 x 32 µl • R 310 15.1 L: 5 x 96 µl • Contains DNA-free Taq DNA Polymerase and Uracil-DNA Glycosylase (heat labile) for prevention of carry-over contamination. • Store at -15 to -25 °C.
3 white cap	**food**proof *Enterobacteriaceae* plus *Cronobacter* Internal Control -LC 2.0	• R 310 15.1: 3 x 32 µl • R 310 15.1 L: 5 x 96 µl • Contains a stabilized solution of plasmid DNA. • For use as an internal amplification control using LightCycler® 2.0. • Store at -15 to -25 °C. • After first thawing store at +2 °C to +8 °C for up to one month.
4 violet cap	**food**proof *Enterobacteriaceae* plus *Cronobacter* Control Template	• R 310 15.1 : 1 x 50 µl • R 310 15.1 L: 1 x 100 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_2O PCR-grade	• R 310 15.1 and R 310 15.1 L: 1 x 1 ml • Nuclease-free, PCR-grade H_2O. • For use as a PCR run negative control. • Store at -15 to -25 °C.
6 black cap	**food**proof *Enterobacteriaceae* plus *Cronobacter* Internal Control -LC 480	• R 310 15.1: 3 x 32 µl • R 310 15.1 L: 5 x 96 µl • Contains a stabilized solution of plasmid DNA and a yellow dye for better visualization. • For use as an internal amplification control using LightCycler® 480. • Store at -15 to -25 °C. • After first thawing store at +2 °C to +8 °C for up to one month.

Storage and Stability
• Store at –15 °C to –25 °C through the expiration date printed on the label.
• Once opened, store the components as described in the following Contents table:

Additional Equipment and Reagents Required
• LightCycler® Carousel-Based System 2.0 Instrument[2]
• LightCycler® 20 µl - Capillaries[2]
• Color Compensation Set 1[1] (Cat. No. A 500 08)
• 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[2] for use with the LightCycler® 2.0 Sample Carousel (optional).
or
• LightCycler® 480 I or II System[2]
• LightCycler® 480 compatible PCR plate and sealing foil[2]
• **food**proof StarPrep One Kit[1] (Cat. No. S 400 07 or Cat. No. S 400 07 L)
• **food**proof Magnetic Preparation Kit IV (Order No. S 400 15)[2]
• Reagent D[1] (Cat. No. A 500 02 or Cat. No. A 500 02 L, recommended)
• Nuclease-free, aerosol-resistant pipette tips
• Pipettes
• 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 **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit is intended for the rapid detection of DNA of *Enterobacteriaceae* isolated from enrichment cultures prepared by valid methods and inoculated with all kinds of foods that are potentially contaminated with these microorganisms. The Detection Kit allows in addition the specific identification of *Cronobacter* spp.. The kit has been MicroVal validated in combination with the **food**proof StarPrep One Kit (S 400 07) and Reagent D (A 500 02) with the ISO methods for *Enterobacteriaceae* and for *Cronobacter* spp.. In samples with a very high content of non-*Cronobacter Enterobacteriaceae* (Cp-Value approx. < 10-15 in channel 640) low amounts of *Cronobacter* DNA might not be detected. For safe detection of *Cronobacter* in such cases the *Cronobacter* Detection Kit (R 310 13) has to be used.

The Detection Kit must not be used in diagnostic procedures.

The Detection Kit described in this Manual Instruction has been developed for the LightCycler® 2.0 Carousel-Based System and the LightCycler® 480 System I or II (96-well block type).

2. How to Use this Product

2.1 Before You Begin

Precautions

Detection of *Enterobacteriaceae* DNA using the **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit requires DNA amplification by PCR. The detection system provides all reagents required for PCR. 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 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 the risk of carry-over contamination. Use a PCR-hood for all pipetting steps.

Keep the **food**proof *Enterobacteriaceae* plus *Cronobacter* 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 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

Positive Control
Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the provided control DNA [Control Template (vial 4, purple cap)] or with a positive sample preparation control.

Negative Control
Always run a negative control with the samples. To prepare a negative control, replace the template DNA with H_2O 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.

Color Compensation
The use of a previously generated color compensation object is a prerequisite for the unambiguous discrimination of *Enterobacteriaceae* DNA, *Cronobacter* DNA and internal control (IC) DNA amplification in this multi-color experiment. A suitable color compensation object can be generated using dedicated reagents available as “Color Compensation Set 1” (Cat. No. A 500 08). 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 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 System Protocol

The following procedure is optimized for use with the LightCycler® 480 System. Program the LightCycler® 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

LightCycler® 2.0 Carousel-Based System Protocol

The following procedure is optimized for use with the LightCycler® 2.0 Carousel-Based System. Program the LightCycler® before preparing the reaction mixes. Use the following LightCycler® 2.0 PCR-program for the **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit (for details on how to program the experimental protocol, see the LightCycler® 2.0 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]	37	95
Hold/Incubation Time [h:min:s]	00:04:00	00:02:00
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			
Programs/Cycle Program Data	***Value***		
Cycles	34		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target/Target Temperature [°C]	95	64	72
Hold/Incubation Time [h:min:s]	00:00:02	00:00:35	00:00:20
Ramp Rate/Temperature Transition Rate [°C/s]	20	20	20
Sec Target/Secondary Target Temperature [°C]	0	60	0
Step Size [°C]	0.0	0.2	0.0
Step Delay [cycles]	0	8	0
Acquisition Mode	None	Single	None

Cooling (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

LightCycler® 2.0 Fluorescence and Run Setup Parameters

Parameter	Setting
All LightCycler® Software Versions	
Seek Temperature	30°C
LightCycler® Software Version 4.x	
Default channel • during run • for analysis	 • Fluorescence channel 640, 670 or 610 • 640/Back 530, 670/Back 530 or 610/Back 530
Fluorescence Gains	not required
“Max. Seek Pos”	Enter the number of sample positions the instrument should look for.
“Instrument Type”	“6 Ch.”: for LightCycler® 2.0 Instrument (selected by default).
“Capillary Size”	Select “20 µl” as the capillary size for the experiment.

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 *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 2.0, (vial 3, white cap) or **food**proof® *Enterobacteriaceae* plus *Cronobacter* Internal Control - LC 480, (vial 6, black cap)	1 µl
Total volume	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_2O PCR-grade (vial 5, colorless cap), seal the capillary with a stopper.
   • For the positive control, add 5 µl **food**proof *Enterobacteriaceae* plus *Cronobacter* 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 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 640/Back-530 (LC 480 I: 483-640, LC 480 II: 498-640), 670/Back-530 (LC 480 I: 483-670, LC 480 II: 498-660) and 610/Back-530 (LC 480 I: 483-610, LC 480 II: 498-610) using the Qualitative Detection module of the LightCycler® Analysis Software for LightCycler® 2.0 and the Absolute Quantification module for the LightCycler® 480 Systems. The instrument- and assay-specific color compensation object (s. section 2.1) must be activated for all channels in order to compenate for crosstalk between the detection channels. Check for a positive result of the Internal Control (visible signal in the channel for 610 nm detection) for each sample that is negative for *Enterobacteriaceae* and *Cronobacter* DNA (no signal in the channels for 640 and 660/670 nm detection). Compare the results for each sample, and interpret as described in the table below:

Enterobacteriaceae **Channel 640**	***Cronobacter*** **Channel 660/670**	**Internal Control Channel 610**	**Result Interpretation**
Positive	Positive	Positive OR Negative	Positive for *Enterobacteriaceae* AND *Cronobacter**
Positive	Negative	Positive Or Negative	Positive for *Enterobacteriaceae*, negative for *Cronobacter*
Negative	Negative	Positive	Negative for *Enterobacteriaceae* AND *Cronobacter*
Negative	Negative	Negative	**Invalid result**

*In case of very small amounts of *Cronobacter* DNA it can happen that the *Cronobacter* channel (660/670) gives a positive signal, whereas the channel for *Enterobacteriaceae* (640) is negative. This indicates a positive result for both *Enterobacteriaceae* and *Cronobacter* and is due to a slightly higher sensitivity of the detection system for *Cronobacter*.

Notes:

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

3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	For Carousel-based LightCycler: Set Channel settings to 610, 640 or 670. 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. For LC 480: Set channel settings to 610, 640 or 660/670.
	Pipetting errors or omitted reagents.	• 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.	• Check the cycle programs. • Select acquisition mode "single" at the end of each annealing segment of the PCR program.
No signal increase in channel 610 is observed.	Inhibitory effects of the sample material (*e.g.,* caused by insufficient purification).	• Use the recommended DNA sample preparation kit to purify template DNA. • Dilute samples or pipet a lower amount of sample DNA (*e.g.,* 2 µl instead of 5 µl). • Perform a sub-cultivation of the enrichment culture (*e.g.,* 1:10 in Buffered Peptone Water) to dilute the portion of food matrix in the sample.
Fluorescence intensity is too low.	In appropriate storage of kit components.	• Store the **food**proof *Enterobacteriaceae* plus *Cronobacter* Master Mix (vial 1, yellow cap) at -15 °C to –25 °C, protected from light. • Avoid repeated freezing and thawing.
	foodproof *Enterobacteriaceae* plus *Cronobacter* Master Mix (vial 1, yellow cap) or the complete PCR mix is not homogeneously mixed.	Mix the **food**proof *Enterobacteriaceae* plus *Cronobacter* Master Mix (vial 1, yellow cap), all other kit components and the complete PCR mix 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.	• Exchange all critical solutions. • Repeat the complete experiment with fresh aliquots of all reagents. • Always handle samples, detection system components and consumables in accordance with commonly accepted practices to prevent carry-over contamination. • For Carousel-based LightCycler: Add positive controls after sample capillaries and negative control capillaries have been sealed with stoppers.
Fluorescence intensity varies.	Insufficient centrifugation of the capillaries or plate. For Carousel-based LightCycler: Prepared PCR mix is still in the upper vessel of the capillary. Air bubble is trapped in the capillary tip.	Always centrifuge capillaries or plates (loaded with the reaction mix) as described.
	For Carousel-based LightCycler: Outer surface of the capillary tip is dirty (*e.g.*, by direct skin contact). For LightCycler 480: Surface of the sealing foil is dirty (*e.g.,* by direct skin contact).	Always wear gloves when handling the capillaries or plates.

4. Additional Information on this Product

How this Product Works

The **food**proof *Enterobacteriaceae* plus *Cronobacter* 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 detection system and to prevent misinterpretation of negative results due to inhibition of the amplification, an Internal Control (IC) is supplied (vial 3 and vial 6, respectively). The IC has to be added to each reaction. Hybridization Probes were designed to bind specifically the IC, allowing detection in channel 610, whereas the *Enterobacteriaceae* DNA is detected in channel 640 and *Cronobacter* DNA in channel 670 or 660 for LC 480 II, respectively. 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 *Enterobacteriaceae/Cronobacter* DNA in the sample. The **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit minimizes contamination risk and contains all reagents (except for template DNA) needed for detection. The detection kit described in this Manual Instruction has been developed for the LightCycler® 2.0 Carousel-Based System and the LightCycler® 480 Systems.

Test Principle

1. Using the product's supplied sequence-specific primers in a polymerase chain reaction (PCR), the LightCycler® and its associated reagents amplify and simultaneously detect specifically DNA of *Enterobacteriaceae/Cronobacter*.
2. The LightCycler® System detects these amplified fragments in real time through fluorescence generated by their corresponding pair of sequence-specific 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® excites the donor fluorophore and part of the excitation energy is transferred to the acceptor fluorophore.
4. The LightCycler® System 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 *Enterobacteriaceae* genomic DNA) does not contain uracil and is therefore not degraded by this procedure.

Product Characteristics

Specificity: In/ex-clusivity of the **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit has been tested with 160 *Cronobacter* spp. strains comprising strains of all genogroups (*Cronobacter sakazakii*, *C. malonaticus, C. turicensis, C. muytjensii, C. dublinensis* and *C.* genomospecies 1) and all 16 biogroups, more than 120 non-*Cronobacter* strains of the family *Enterobacteriaceae* comprising 61 species (including the most closely related *Enterobacter helveticus* and *E. pulveris*) as well as more than 60 non-*Enterobacteriaceae* species (mostly of the closely related genera like *Aeromonas* or *Vibrio)*.
All *Cronobacter* spp. strains were detected in channel 640 and 660/670, all non-*Cronobacter Enterobacteriaceae* in channel 640 and none of the non-*Enterobacteriaceae* strains were detected in any channel.

Sensitivity: A relative detection limit of 1 to 10 cells per 25/100 g sample can be achieved with all relevant kinds of foods. The **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit detects down to 10^3 - 10^4 cfu/ml of *Enterobacteriaceae/Cronobacter* enrichment culture (depending on the sample preparation kit used).

References

1. C. Grönewald, M. Kiehne, K. Berghof-Jäger, Hygiene Report 1-2006, 22

Quality Control

The **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit is function tested using the LightCycler® Carousel-Based System and the LightCycler® 480 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

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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 1, April 2015
The name of the kit has been changed from **food**proof *Enterobacteriaceae* plus *E. sakazakii* Detection Kit to **food**proof *Enterobacteriaceae* plus *Cronobacter* Detection Kit due to taxonomically reasons.

Version 2, April 2016
Information about Large version of the kit with 480 reactions has been added.

Version 3, March 2017
License Notice changed.

Version 4, September 2017
License Notice changed.

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