FOR *IN VITRO* USE ONLY

foodproof® *Vibrio* Detection LyoKit – 5´Nuclease –

Version 2, March 2017

PCR kit for the qualitative detection of *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio cholerae* and their associated toxin genes *tdh*, *trh1*, *trh2* and *ctx* using real-time PCR instruments.

Order No. R 602 44-1 / R 602 44-2

Kit for 96 reactions (lyophilized)
for a maximum of 94 samples
Store the kit at 2 to 8 °C

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1. What this Product Does

Number of Tests

The kit is designed for 96 reactions with a final reaction volume of 25 µl each. Up to 94 samples (single sample preparation) plus positive and negative control reactions can be analyzed per run.

Storage and Stability

- Store the kit at 2 °C to 8 °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

Component	Label	Contents / Function / Storage
foodproof® *Vibrio* Detection LyoKit Microplate, prefilled with 96 reactions (lyophilized)	Aluminum bag containing an 8-tube strip mat • R 602 44-1 with white low profile tubes* • R 602 44-2 with clear regular profile tubes*	• 96 prefilled reactions (lyophilized). • Ready-to-use PCR mix containing primer and probes specific for DNA of the three major pathogenic *Vibrio* species and the Internal Control (IC) as well as Taq DNA Polymerase and Uracil-N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination. • For amplification and detection of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* • For amplification and detection of pathogenicity associated genes *thermostable direct hemolysin* (*tdh*), *tdh-related hemolysin* (*trh1* and *trh2*) and *cholera toxin* (*ctx*) • Store at 2 °C to 8 °C in the aluminum bag (sealed). • **Protect from light and moisture!**
Control Template	Vial 2 (purple cap)	• 1 x 350 µl • Contains a stabilized solution of DNA. • For use as a PCR run positive control. • Store at 2 to 8 °C.
H_2O PCR-grade	Vial 3 (colorless cap)	• 1 x 1 ml • Nuclease-free, PCR-grade H_2O. • For use as a PCR run negative control.
Cap strips	Plastic bag containing 8-cap strips	• 12 x 8-cap strip • For use in real-time PCR after addition of samples.

***Tube profile and instrument compatibility chart is available online: www.bc-diagnostics.com/compatibility-chart

Additional Equipment and Reagents Required

- Real-time PCR cycler suitable for detection of FAM-, HEX-, ROX- and Cy5-labeled probes and capable of performing a melting curve analysis. Without a melting curve analysis, the *Vibrio* species can still be detected but the presence of toxin genes cannot be assessed. In cases the strip tubes do not fit for the instrument the samples have to be transferred to appropriate PCR vessels after resuspension of the lyophilized PCR mix.
- Sample Preparation Kit
  foodproof® StarPrep Three Kit (Order No. S 400 18)[1]
  Reagent D (small version: Order No. A 500 02 S)[1]
- Nuclease-free, aerosol-resistant pipette tips
- Pipettes
- Vortex centrifuge Multispin MSC-6000 for PCR-strips (Order No. D 110 66)[1] **with**
- SR-32, Rotor for MSC-3000/6000 (Order No. D 110 65)[1] **or**
- Vortex centrifuge CVP-2 for PCR-plates (Order No. D 110 67)[1]

[1] Available from BIOTECON Diagnostics; see ordering Information for details

Applicability Statement

The **food**proof® *Vibrio* Detection LyoKit – 5'Nuclease – is intended for the qualitative detection of the three major human pathogenic *Vibrio* species *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae,* isolated from enrichment cultures prepared by valid methods with all relevant kinds of samples that are potentially contaminated with these microorganisms, e.g. seafood. In addition, presence of the pathogenicity associated toxin genes *thermostable direct hemolysin* (*tdh*), *tdh-related hemolysin* (*trh1* and *trh2*) and *cholera toxin* (*ctx*) in isolates may be qualitatively assessed by melting curve analysis.

The enrichment can be done for 16 to 18 hours in media according to e.g. ISO/TS 21872-1&2:2007 (Alkaline Saline Peptone Water, ASPW) or FDA BAM Chapter 9 (Alkaline Peptone Water, APW). For the AOAC validation, 25 g samples were enriched in APW for 16 to 18 hours at 36 ± 1 °C. For raw shrimps, 6 to 8 hours can also be used. DNA extraction was performed using StarPrep Three and Reagent D, according to the respective package inserts.

To be used in the analytical laboratory for food, bioburden and environmental testing applications. The kit must not be used in diagnostic procedures.

The kit described in this Instruction Manual has been developed for real-time PCR instruments with a FAM, a HEX, a ROX and a Cy5 detection channel which are capable of performing a melting curve analysis. For the AOAC RI validation the performance of the kit was tested with the following real-time PCR instruments: LightCycler® 480, LightCycler® 96 (Roche Diagnostics), AriaMx (Agilent Technologies), ABI 7500 Fast (Applied Biosystems) and CFX96™ (Bio-Rad). In addition, the PikoReal® 24 (Thermo Fisher Scientific) and the Mx3005P® (Agilent Technologies) were tested during the internal validation.

Note: A Color Compensation is necessary and will be supplied by BIOTECON Diagnostics for users of the LC 480 System I (Color Compensation Set 3; Order No. A 500 10) and LC 480 System II (Color Compensation Set 5; Order No. A 500 15).
For users of the Applied Biosystems 7500 fast the "7500 Fast System SDS software" is used for data interpretation (for further information, please contact BIOTECON Diagnostics).

2. How to Use this Product

2.1 Before You Begin

Precautions and Safety

Detection of DNA using the **food**proof® *Vibrio* Detection LyoKit requires DNA amplification by PCR. The kit provides all reagents required for the PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions. For all work with Vibrio species risk group 2 facilities are adequate. Follow the instructions below to avoid nuclease-, carry-over-, or cross-contamination:

- **Keep the kit components separate** from other reagents in the laboratory.
- Use **nuclease-free labware** (*e.g.,* pipettes, pipette tips, reaction vials).
- The **use of gloves** is recommended for all handling of raw material and food samples, DNA samples, and all handling of PCR reagents.
- 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.
- A **safety cabinet** is recommended for all work with potentially aerosol forming microbiological material, as well as appropriate disinfection arrangements (disinfectant dispenser etc.) and deactivation of arising waste by autoclaving.
- Physically **separate the workplaces** for DNA preparation, PCR setup, and PCR to minimize the risk of carry-over contamination. Use a PCR hood for all pipetting steps.

BIOTECON Diagnostics recommends following the German DIN norm 58956 for safety regulations and safety engineering systems of medical and microbiological laboratories while carrying out all portions of the test kit.

Keep the foodproof® ***Vibrio*** **Detection lyophilized PCR Mix away from light and moisture.**

Sample Material

Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors. For preparation of genomic DNA from various sample 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 food samples and PPS (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 [**food**proof® *Vibrio* Detection Control Template (vial 2, 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 3, 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.

2.2 Procedure

Program Setup

The following procedure is optimized for a real-time PCR instrument with a FAM (amplification of *Vibrio parahaemolyticus*, melting curve identification of *tdh*), HEX (amplification of *Vibrio vulnificus*, melting curve identification of *trh1* and *trh2*), ROX (amplification of *Vibrio cholerae*, melting curve identification of *ctx*) and Cy5 (amplification of the Internal Control) detection channel. Program the PCR instrument before preparing the PCR samples. Use the following real-time PCR-protocol for the **food**proof® *Vibrio* Detection LyoKit. For details on how to program the experimental protocol, see the Instrument Operator's Manual of your real-time PCR-cycler:

Pre-incubation	**1** cycle
Step 1:	37 °C for 4 minutes
Step 2:	95 °C for 5 minutes
Amplification	**50** cycles
Step 1*:	95 °C for 5 seconds
Step 2**:	60 °C for 60 seconds

* Set time to 10 seconds on the Agilent Mx3005P instrument
** Fluorescence detection in step 2

Melting Curve	**1** cycle
Step 1:	95 °C for 50 seconds
Step 2:	37 °C for 50 seconds
Step 3*:	ramp up to 65 °C

* Fluorescence detection during 37 – 65 °C ramp with 2 – 4 measurements/°C

Notes:

- For some real-time PCR instruments the type of the probe quencher as well as the usage of a passive reference dye has to be specified. The **food**proof® *Vibrio* Detection LyoKit contains probes with a non-fluorescent ("dark") quencher and no passive reference dye.
- For users of the Agilent Mx3005P instrument: Choose Experiment Type "SYBR® Green (with Dissociation Curve)" and add HEX, ROX, and CY5 channels for data collection in the setup section.
- For users of the Agilent Mx3005P instrument: Click "Instrument → Filter Set Gain Settings" to open the Filter Set Gain Settings dialog box in which the gain settings may be viewed and modified. For FAM and HEX the Filter Set Gain Setting must be modified to "x4". For ROX and Cy5 the Filter Set Gain Setting must be modified to "x1".
- Please contact BIOTECON Diagnostics, if you have questions how to program your cycler

Preparation of the PCR Mix

Proceed as described below to prepare a 25 µl standard reaction. Always wear gloves when handling strips or caps. Use any sample material suitable for PCR in terms of purity, concentration, and absence of inhibitors.

Note: The PCR strips must be stored in the provided aluminum bag with the silica gel pads to avoid liquid absorption.

1. Take the needed number of PCR tube strips out of the aluminum bag. Use scissors or scalpel to cut the strips apart. Tightly seal the bag afterwards and store away at the recommended conditions.
2. Place the PCR tube strips containing the lyophilized reagents in a suitable PCR tube rack. Check that the reagent pellets are at the bottom of the tubes. If not, briefly centrifuge or flick the pellets to the bottom before proceeding.
3. Decap the tube strips cautiously and discard the cap strips.

Note: Do not leave strips open for extended periods of time. To avoid unwanted liquid absorption, open strips only shortly before filling.

4. Pipet 25 µl sample into each PCR-vessel:
   - For the samples of interest, add 25 µl sample DNA (if using less volume, add PCR-grade H_2O to achieve 25 µl).
   - For the negative control, add 25 µl PCR-grade H_2O (vial 3, colorless cap).
   - For the positive control, add 25 µl **food**proof® *Vibrio* Detection Control Template (vial 2, purple cap).

Note: To reduce the risk of cross-contamination, it is recommended to prepare only one PCR tube strip at a time.

5. Seal the vessels accurately and tightly with the colorless cap strips.
6. Mix thoroughly using a vortex centrifuge.

Note: BIOTECON Diagnostics recommends vortex centrifuges Multispin MSC-3000 (D 110 64) for PCR-strips or vortex centrifuge CVP-2 for PCR-plates (D 110 67). Dedicated protocols are available for this centrifuge.

Note: Alternatively resuspend the pellet by manual mixing. This may be achieved by cautiously pipetting the sample up and down multiple times during step 4 or flipping the tube strips after sealing while pressing down the cap strip.

7. Spin the PCR tube strips for 30 seconds at 150 – 200 g in a suitable centrifuge.

Note: If your centrifuge exceeds 200 g, do not centrifuge for more than 5 seconds. Avoid centrifugation at forces exceeding 1000 g!

8. Place the samples in your PCR cycler and run the program as described above.

Note: For using any LightCycler 480 instrument, a special adapter (Order No. Z 100 24) is necessary. For some PCR instruments, the PCR strips should be placed in a balanced order into the cycler block. For example two strips can be placed in column 1 and 12.

2.3 Data Interpretation

The kit is intended for the qualitative detection of *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae* as well as their associated toxin genes *tdh*, *trh1*, *trh2* and *ctx*.

Amplification

The amplification of *Vibrio parahaemolyticus* is analyzed in the fluorescence channel suitable for FAM labeled probes detection. The amplification of *Vibrio vulnificus* is analyzed in the fluorescence channel suitable for HEX. The amplification of *Vibrio cholerae* is analyzed in the fluorescence channel suitable for ROX. The amplification of the **Internal Control** is analyzed in the fluorescence channel suitable for Cy5.
Compare the results from channels FAM, HEX and ROX for each sample, and interpret the results as described in the table below.

Channel FAM	Channel HEX	Channel ROX	Channel Cy5	Result Interpretation
Positive	Positive or Negative	Positive or Negative	Positive or Negative	Positive for *V. parahaemolyticus*
Positive or Negative	**Positive**	Positive or Negative	Positive or Negative	Positive for *V. vulnificus*
Positive or Negative	Positive or Negative	**Positive**	Positive or Negative	Positive for *V. cholerae*
Negative	**Negative**	**Negative**	**Positive**	Negative for the above *Vibrio*
Negative	**Negative**	**Negative**	**Negative**	Invalid

Note: The Control Template contains a mixture of all target sequences and therefore usually generates significantly higher fluorescent values than samples that are positive for only one or two of the targets. This can affect positive/negative calls in automatic analysis of amplification curves by the respective instrument software. Always check results visually for plausibility.

Melting curves

Samples that show a positive amplification signal in the FAM or ROX detection channel can be further differentiated using a melting curve analysis. A prerequisite for the unambiguous detection and discrimination of the *Vibrio* toxin types in this multi-color experiment is a suitable calibration of the PCR instrument for channels FAM, HEX and ROX. Please refer to the Operator's Manual of your real-time PCR cycler for further information.

The following table lists the detectable *Vibrio* toxin types in the respective channels and their expected melting peak temperatures (± 2 °C dependent on the real-time PCR instrument):

***Vibrio* toxin**	**FAM channel – melting peak temperature**	**HEX channel – melting peak temperature**	**ROX channel – melting peak temperature**
tdh [1]	**49 °C** ± 2 °C	none	none
trh1 [1]	none	**53.5 °C** ± 2 °C	none
trh2 [1]	none	**47 °C** ± 2 °C	none
ctx [2]	none	none	**49 °C** ± 2 °C
Control Template	**49 °C** ± 2 °C	**53.5 °C** ± 2 °C	**49 °C** ± 2 °C

Note: The melting peak temperature ranges given in the above table mainly reflect the variability between instruments and their respective analysis software. The Control Template contains a mixture of all target sequences except *trh2*. Functionality of *trh2* detection is provided by the *trh1* melting curve. Mind that melt curves in channel HEX are associated with *Vibrio parahaemolyticus* (detected in channel FAM). [1] Associated with pathogenic *Vibrio parahaemolyticus*. [2] Associated with pathogenic *Vibrio cholerae*. *V. vulnificus* does not contain any of the toxin genes detected with this kit.

Melting peaks can occur at a temperature greater than 60 °C. These are not attributed to one of the target toxin types and can safely be ignored.

The peak height of positive samples may vary according to the initial cell concentration. Melting peaks of positive samples may be absent, if target is detected with an amplification cycle later as 30. If the melting curve is absent at an amplification cycle later as 30, please subcultivate the sample 1:10 in APW at 37 °C for 12 to 18 hours, and repeat DNA extraction and PCR. Note that the presence or absence of specific melting peaks should be checked manually for all positive samples as the peak finding algorithms of the respective PCR instrument software may not detect all relevant maxima of the melting curve. A guarantee for the identification via melting curves cannot be given.

3. Troubleshooting

Observation	Possible Reason	Recommendation
No signal increase is observed, even with positive controls.	Incorrect detection channel has been chosen.	• Set channel settings to FAM.
	Pipetting errors.	• Check for correct reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.
	No data acquisition programmed.	• Check the cycle programs.
No signal increase in channel Cy5 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.,* 20 µl PCR-grade H_2O and 5 µl sample DNA instead of 25 µl sample DNA).
Fluorescence intensity is too low.	Inappropriate storage of kit components.	• Store the **food**proof® *Vibrio* Detection lyophilized PCR Mix at 2 °C to 8 °C, protected from light and moisture.
	Low initial amount of target DNA.	• Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.
Strong decrease of fluorescence baseline	Resuspension of lyophilized PCR mix not complete.	• Always resuspend lyophilized PCR mix thoroughly.
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, kit components and consumables in accordance with commonly accepted practices to prevent carry-over contamination.
Fluorescence intensity varies.	Insufficient centrifugation of the PCR strips. Resuspended PCR mix is still in the upper part of the vessel.	• Always centrifuge PCR strips.
	Outer surface of the vessel or the seal is dirty (*e.g.,* by direct skin contact).	• Always wear gloves when handling the vessels and seal.
Pellets are difficult to dissolve.	The lyophilized PCR mix started to rehydrate.	• Store the lyophilized PCR mix always tightly sealed in the aluminum bag with the silica gel pad. • Open strip shortly before filling.

4. Additional Information on this Product

How this Product Works

The **food**proof® *Vibrio* Detection LyoKit provides all necessary 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 included. A hydrolysis probe was designed to bind specifically the IC, allowing detection in the Cy5 channel, whereas the *Vibrio* DNA is detected in channels FAM, HEX and ROX. In 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 DNA of the target organisms in the sample. The **food**proof® *Vibrio* Detection LyoKit minimizes contamination risk and contains all reagents (except for template DNA) needed for the detection of DNA of the target organisms and toxins. Primers and probes provide specific detection in food samples. The described performance of the kit is guaranteed for use on the real-time PCR instruments listed above only.

Test Principle

1. Using the kit's sequence-specific primers in a polymerase chain reaction (PCR), the PCR instrument and the supplied reagents amplify DNA fragments specific for human pathogenic *Vibrio* and their associated toxins.
2. The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probes due to the 5´-nuclease activity of the Taq DNA polymerase.
3. During the ramp phase of the PCR (melting curve), additional probes hybridize to an internal sequence of the amplicons which separates the reporter dye from the quencher dye, increasing the reporter dye signal.
4. The PCR instrument measures the emitted fluorescence of the reporter dye.

Prevention of Carry-Over Contamination

The heat-labile Uracil-N-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 *Vibrio* 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 **food**proof® *Vibrio* Detection LyoKit, decontamination can be achieved with the provided reagents.

Background Information

Members of the genus *Vibrio* are defined as Gram-negative, asporogenous rods that are straight or have a single, rigid curve. Three species, *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, are well-documented human pathogens. *Vibrio* species account for a significant proportion of human infections from the consumption of raw or undercooked shellfish. A study of illnesses from raw shellfish consumption reported the following species in descending order of frequency; *V. parahaemolyticus*, *non-O1/O139 V. cholera* (*ctx*-negative), *V. vulnificus*, *O1 V. cholerae* (*ctx*-positive) [1]. Health associated risks differ among human pathogenic *Vibrio*: *V. parahaemolyticus* has been reported to be responsible for one-fourth of all gastrointestinal pathologies caused by food. In the United States, it caused 14 food-poisoning outbreaks between 1971 and 1978. Though *Vibrio vulnificus* does not cause outbreaks, it is responsible for very severe syndromes that are fatal in 50-60%of cases [2]. *V. cholerae*, is the causative agent of cholera outbreaks and epidemics. *V. cholerae* strains that are identical to, or closely resemble, clinical strains in biochemical characteristics, but fail to agglutinate in either anti-O1 or -O139 sera are now referred to as *V. cholerae* non-O1/O139. These serologically diverse strains are abundant in estuarine environments. Evidence indicates that non-O1/O139 strains are sporadically involved in cholera-like diarrheal disease [1].

Several virulence factors are closely associated with the pathogenic potential of *Vibrio* isolates:

tdh, *trh1* and *trh2* genes (*Vibrio parahaemolyticus*)

Most clinical isolates of *V. parahaemolyticus* are differentiable from environmental strains by their ability to produce a thermostable direct hemolysin (TDH), termed the Kanagawa phenomenon. A thermostable related hemolysin (TRH), which shares 60 % homology with TDH, has also been associated with strains causing gastroenteritis. Presently, there is no in vitro test to detect TRH production. Many clinical strains of *V. parahaemolyticus* produce both TDH and TRH [1]. Most environmental isolates do not contain *tdh* or *trh* genes [3].

ctxA and *ctxB* genes (*Vibrio cholerae*)

Cholera enterotoxin (CTX) is the primary virulence factor of the disease cholera. Most *V. cholerae* strains recovered from epidemic cholera cases contain a common somatic antigen and include serogroup O1. Until recently, only the O1 serogroup was associated with cholera epidemics. However, in 1993, a large outbreak of cholera occurred in India/Bangladesh from a new, until then unknown serogroup, O139 [1].

Detection of human pathogenic *Vibrio* and associated toxin genes with the **food**proof® *Vibrio* Detection LyoKit is in accordance with US FDA Bacteriological Analytical Manual methods [1].

References

1. Kaysner CA and DePaola A Jr., BAM “Chapter 9: *Vibrio”*, May 2004, FDA Bacteriological Analytical Manual, 8th Edition, Revision A; with modifications
2. Flick GJ Jr., “Pathogenic Vibrios In Shellfish”, Global Aquaculture Advocate, 2007 Nov-Dez, 46-48
3. Drake SL, DePaola A, Jaykus LA, “An Overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*”, Comprehensive Reviews in Food Science and Food Safety, 2007, Vol6:4, 120-144

Quality Control

The **food**proof® *Vibrio* Detection LyoKit is function tested using the LightCycler® 480 System (R 602 44-1) and the Mx3005P® (R 602 44-2).

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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5.4 Contact and Support

If you have questions about 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, July 2015
First version of the package insert.
Version 2, March 2017
License Notice changed.
Introduction of vortex centrifuges into the PCR Setup Procedure.

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