Foodproof® GMO Bt176 Maize Quantification Kit – 5’Nuclease –

Version 2, March 2017

PCR kit for the quantitative detection of genetically modified maize event Bt176 using real-time PCR instruments

Order No. R 302 16

Kit for 128 reactions for a maximum of 48 – 60 samples

Store the kit at -15 to -25 °C
1. Product Overview

Number of Tests

The kit is designed for 128 reactions [i.e., 64 reactions with the GMO Gene Master Mix, (vial 1, yellow cap) and 64 reactions with the Reference Gene Master Mix, (vial 2, green cap)] with a final reaction volume of 25 µl each.

Note: The maximum number of samples that can be analyzed per experiment depends on the chosen quantification procedure:

<table>
<thead>
<tr>
<th>Procedure A</th>
<th>Procedure B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Including relative standard curves</td>
<td>Importing external relative standard curves</td>
</tr>
<tr>
<td>Quantification of up to 48 samples (single sample preparation) in two runs plus calibration curves, one negative control reaction, and one calibrator in each run.</td>
<td>Quantification of up to 60 samples (single sample preparation) in two runs plus calibrator and one negative control reaction in each run.</td>
</tr>
</tbody>
</table>

Storage and Stability

- Store the kit at –15 °C to –25 °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

<table>
<thead>
<tr>
<th>Vial</th>
<th>Label</th>
<th>Contents / Function / Storage</th>
</tr>
</thead>
</table>
| 1 yellow cap | foodproof GMO Bt176 Maize - GMO Gene Master Mix - | 2 x 420 µl  
- Ready-to-use primer and 5'-nuclease probe mix specific for the crylA(b) gene of genetically modified Bt176 maize.  
- For amplification and detection of the crylA(b) gene.  
- Store at -15 to –25 °C.  
- Avoid repeated freezing and thawing!  
- Protect from light! |
| 2 green cap | foodproof GMO Bt176 Maize - Reference Gene Master Mix - | 2 x 420 µl  
- Ready-to-use primer and 5'-nuclease probe mix specific for the starch synthase gene (zSSIIb) of native maize  
- For amplification and detection of the zSSIIb gene.  
- Store at -15 to -25 °C.  
- Avoid repeated freezing and thawing!  
- Protect from light! |
| 3 red cap | foodproof GMO Bt176 Maize - Enzyme Solution - | 4 x 32 µl  
- Contains Taq DNA Polymerase and Uracil-DNA N-Glycosylase (UNG, heat labile) for prevention of carry-over contamination.  
- Store at -15 to -25 °C. |
| 4 black cap | foodproof GMO Bt176 Maize - Dye Solution - | 4 x 32 µl  
- Contains a yellow dye for better visualization of the PCR mix in white PCR plates. |
| 5 purple cap | foodproof GMO Bt176 Maize - Calibrator DNA - | 2 x 50 µl  
- Contains a stabilized solution of plasmid DNA.  
- For use as a PCR run calibrator and positive control.  
- Store at -15 to -25 °C.  
- After first thawing store at +2 °C to +8 °C for up to one month. |
| 6 blue cap | foodproof GMO Bt176 Maize - Dilution Buffer - | 4 x 1 ml  
- For dilution of calibrator and sample DNA.  
- Store at -15 to -25 °C. |
| 7 colorless cap | foodproof GMO Bt176 Maize - H₂O PCR-grade - | 2 x 1 ml  
- Nuclease-free, PCR-grade H₂O.  
- For use as a PCR run negative control.  
- Store at -15 to -25 °C. |
Product Description
The foodproof GMO Bt176 Maize Quantification Kit provides PCR primers and hydrolysis probes (5’-nuclease probes), and convenient premixed reagents for sequence specific amplification and detection of the cryIA(b) gene (GM-specific DNA) and the maize zSSIIb gene (taxon-specific DNA). A Calibrator DNA is also provided to ensure accurate determination of the DNA copy number ratio of the GM-specific DNA to the taxon-specific DNA, expressed in %.

Results are obtained within 100 minutes. Optimized PCR conditions allow analysis of the GM-specific and the taxon-specific PCR in a single run. The Calibrator DNA provided with the kit serves as positive control and as a reference to normalize the relative DNA copy number ratio. Normalization corrects for differences in GMO content values, resulting from the combined variation in the quantity and quality of DNA samples and the efficiency of the PCR. The foodproof GMO Bt176 Maize Quantification Kit is specifically adapted for PCR using real-time PCR instruments.

• The GMO Gene Master Mix, (vial 1, yellow cap) allows the amplification and detection of a fragment of the synthetic cryIA(b) gene of genetically modified Bt176 maize using specific primers.

• A fragment of the native maize starch synthase gene (zSSIIb) is amplified and detected with the Reference Gene Master Mix (vial 2, green cap). The reaction product serves both as a control for DNA integrity and as a reference for relative quantification.

Note: The kit described in this instruction manual has been developed for real-time PCR instruments.

Test Principle
Bt-176 maize is identified by detecting a DNA fragment of the synthetic cryIA (b) gene, which originates from the bacterium Bacillus thuringiensis. This gene is responsible for crop resistance to the European corn borer. To detect native maize, a maize-specific starch synthase gene (zSSIIb) is amplified and detected with the Reference Gene Detection Mix, which serves as both a control for DNA integrity and as a reference for relative quantification. Both amplicons are detected in separate reactions with specific pairs of primers and hydrolysis probes (5'-nuclease probes) using a real-time PCR instrument.

The basic steps of the test are as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Using the kit’s supplied sequence-specific primers in a polymerase chain reaction (PCR), the real-time PCR instrument and its associated reagents amplify and simultaneously detect fragments of genetically modified Bt176 maize and native maize, respectively.</td>
</tr>
<tr>
<td>2</td>
<td>The PCR instrument detects these amplified fragments in real time through fluorescence generated by cleavage of the hybridized probe due to the 5’-nuclease activity of the Taq DNA polymerase. The probe is labeled at the 5’-end with a reporter fluorophore and at the 3’-end with a quencher.</td>
</tr>
<tr>
<td>3</td>
<td>During the annealing/elongation phase of each PCR cycle, the probe hybridizes to an internal sequence of the amplicon downstream from one of the primer sites and is degraded by the 5’-nuclease activity of the Taq DNA polymerase. This cleavage of the probe separates the reporter dye from the quencher dye, increasing the reporter dye signal.</td>
</tr>
<tr>
<td>4</td>
<td>The real-time PCR instrument measures the emitted fluorescence of the reporter dye.</td>
</tr>
</tbody>
</table>
| 5    | Determination of the relative DNA copy number ratio of the genetically modified Bt176 maize event. This can be performed using one of the following alternative procedures:  
  • Procedure A: Including relative standard curves into the run.  
  • Procedure B: Importing external relative standard curve from a previous run. |

Application
The foodproof GMO Bt176 Maize Quantification Kit is intended for GMO testing purposes only.

The foodproof GMO Bt176 Maize Quantification Kit is used to determine the relative DNA copy number ratio of genetically modified Bt176 maize in flour or food. Relative quantification is performed by comparing the amplification of a specific gene of genetically modified Bt176 maize with the amplification of a specific gene of native maize. For calculation purposes, included or external (imported) standard curves can be used.

Note: The foodproof GMO Bt176 Maize Quantification Kit has been validated to quantify a DNA copy number ratio of up to 5 % GMO content.
Product Characteristics

**Specificity**
The primers and hydrolysis probes (5’-nuclease probes) provided in the GMO Gene Master Mix, (vial 1, yellow cap) and in the Reference Gene Master Mix, (vial 2, green cap) are sequence-specific for Bt176 maize and native maize, respectively.

**Sensitivity**
Detects the relative amount of 0.1% genetically modified Bt176 maize content in raw material.

**Measuring Range**
The kit can measure the relative content of Bt176 maize event in a range of 0-5 % (for standard material containing 0.1 % GMO the coefficient of variation is equal to or less than 50 %; for standard material containing 1-5 % GMO the coefficient of variation is equal to or less than 35 %).

Background Information

Real-time Polymerase Chain Reaction is commonly used to quantify GM fractions in food and feed samples. This DNA-based quantification technique measures the ratio between transgenic deoxyribonucleic acid (DNA), i.e. derived from the genetic modification, and endogenous DNA, which is specific for the biological species. The genetically modified maize “Event 176” or “Maximizer”, better known as Bt176 maize, was the first GMO maize approved in the EU [1]. Bt176 maize carries a gene that codes for the Cry1A(b) toxin, derived from the bacterium *Bacillus thuringiensis*, which confers resistance to the European corn borer. This plant pest leads to yield losses and high costs for crop protection. Additionally, Bt176 maize is herbicide-tolerant to glufosinate ammonium, and carries a marker gene expressing resistance to the antibiotic ampicillin. According to Swiss and EU law [2-3], foods and additives with more than 1% and 0.9% (in Switzerland and in European countries, respectively) relative amount of genetically modified organisms must be labeled as GMO products.

*Note:* The value of the DNA ratio in reference materials may be not the same as the value of the certified powder mass fraction, because of the different genetic composition of different parts of the seeds of monocotyledons (e.g. maize endosperm, seed coat and embryo). The mass fraction of a reference material expressed in g/kg or % takes the zygosity, ploidy and endoreduplication status of the seed used to produce the material not into account. In the European Regulation, the GM content is defined as the percentage of GM DNA copy number in relation to target taxon specific DNA copy number, calculated in terms of haploid genomes [4]. The determination of the GMO content on basis of the number of copies of the target sequences per haploid genome will also be influenced by zygosity of the maize event. This difference is normally expressed as correction factor (c).

2. Procedure
2.1 Before You Begin

**Precautions and Warnings**
Quantification of the relative GMO content using the foodproof GMO Bt176 Maize Quantification Kit requires DNA amplification by PCR. The kit provides all required reagents in a ready-to-use master mix for the performance of PCR. However, in order to achieve reliable results, the entire assay procedure must be performed under nuclease-free conditions:

- Prepare appropriate aliquots of the kit 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.
- 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.
- In order to avoid cross-contamination, close all capillaries that contain sample DNA and negative controls before pipetting positive controls.

*Note:* Protect the GMO Gene Master Mix, (vial 1, yellow cap) and the Reference Gene Master Mix, (vial 2, green cap) from light.
Additional Equipment and Reagents Required

- Real-time PCR instruments with a FAM detection channel
- Real-time PCR compatible capillaries, tubes, strips or plates with optical cap or foil applicable for the PCR-cycler in use
- Standard benchtop microcentrifuge containing a rotor for 2.0 ml reaction tubes.
- Standard swing bucket centrifuge containing a rotor for multiwell plates with suitable adaptors.

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

1 for use with the LightCycler® 2.0 Sample Carousel (optional).

If you use a LightCycler® Instrument version below 2.0 you need in addition the LC Carousel Centrifuge 2.0 Bucket 2.1

To adapt the LightCycler® 2.0 Sample Carousel to the former LC Carousel Centrifuge, you need the LC Carousel Centrifuge 2.0 Rotor Set

1 Available from Roche Diagnostics
2 Available from BIOTECON Diagnostics

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 detection channel. The performance of the kit was tested with the following real-time PCR instrument: LightCycler® 480 II instrument, LightCycler® 2.0 instrument, LightCycler® 1.x instrument (Roche Diagnostics).

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 of plant origin or from food, refer to the corresponding product package inserts of a suitable sample preparation kit (see Additional Equipment and Reagents Required).

Note: In order to quantify the relative amount of genetically modified maize in a sample of interest, the sample DNA must be diluted at least 1:4 in the Dilution Buffer, (vial 6, blue cap) provided with the kit. This dilution step is essential to compensate for the different ion concentrations of the Calibrator DNA and the sample DNA. The latter depends on the buffers used for the sample preparation procedure. This dilution step also reduces the risk of inhibitory effects.

Assay Time

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Setup</td>
<td>15 min</td>
</tr>
<tr>
<td>PCR run</td>
<td>100 min (e.g. LC 480 II)</td>
</tr>
<tr>
<td>Total assay time</td>
<td>115 min</td>
</tr>
</tbody>
</table>

Positive Control

Always run a positive control with the samples. To prepare a positive control, replace the template DNA with the Calibrator DNA, (vial 5, purple cap) or with a positive sample preparation control (e.g. Certified Reference Material).

Negative Control

Always run a negative control with the samples. To prepare a negative control, replace the template DNA with PCR-grade water (vial 7, 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 Program Setup
Program the PCR instrument before preparing the reaction mixes. The amplification is carried out according to the following temperature-time-program (for details on how to program the experimental protocol, see the operation manual of your real-time PCR cycler):

**Program:**

**Pre-incubation**  
1 cycle

**Step 1:** 37 °C for 4 minutes  
**Step 2:** 95 °C for 10 minutes

**Amplification**  
50 cycles

**Step 1:** 95 °C for 15 seconds  
**Step 2:** 63 °C for 60 seconds

Fluorescence detection in step 2

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 determined. The foodproof GMO Bt176 Maize Quantification Kit contains probes with TAMRA as quencher and no passive reference dye.

NOTE 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 the Filter Set Gain Setting has to be modified to “x1”.

2.3 Experimental Setup

**General Remarks**

Determination of the relative ratio content of Bt176 maize can be performed using one of the following alternative procedures:

- Procedure A: Quantification using included (in-run) relative standard curves
- Procedure B: Quantification using external (imported) relative standard curves

Thoroughly read the software instructions prior to performing this assay.

**Procedure A - Quantification using included (in-run) relative standard curves**

Each individual real-time PCR run consists of:

- Six dilution steps of the Calibrator DNA for both the GMO gene and the reference gene PCR in order to generate the respective calibrator curves (see table below),
- A variable number of sample preparations to be analyzed for genetically modified maize DNA amplification,
- At least one negative control reaction to control for contamination of the GMO gene and the Reference Gene PCR Master Mix, respectively.
- One positive reaction with the Calibrator DNA each for the GMO gene and the Reference PCR to compensate for constant differences between the PCR performance of the GMO gene and the reference gene.

Therefore, a typical experiment consists of 16 wells needed for controls, plus 2 × (n) wells needed for the samples of interest, where (n) indicates the number of food samples of interest. Since a multiwell plate has 96 wells, 40 food samples can be analyzed during one PCR run if the GMO gene and the reference gene are analyzed in the same run.

**Dilution of Calibrator DNA**

Quantification of the GMO content via procedure A requires the stepwise dilution of the Calibrator DNA (vial 5, purple cap) in the Dilution Buffer (vial 6, blue cap) as shown below:

<table>
<thead>
<tr>
<th>Dilution step</th>
<th>Dilution</th>
<th>Concentrations to be entered as standards for the Reference gene or the GMO gene PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Undiluted</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>1:4</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>1:16</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>1:54</td>
<td>1.56</td>
</tr>
<tr>
<td>5</td>
<td>1:256</td>
<td>0.39</td>
</tr>
<tr>
<td>6</td>
<td>1:1024</td>
<td>0.098</td>
</tr>
</tbody>
</table>
Procedure B - Quantification using external (imported) relative standard curves
Each individual real-time PCR run consists of:
• One positive reaction with the Calibrator DNA each for the GMO gene and the Reference gene PCR to compensate for constant differences between the PCR performance of the GMO gene and the reference gene,
• A variable number of sample preparations to be analyzed for genetically modified maize DNA amplification,
• At least one negative control reaction to control for contamination of the GMO gene and the Reference Gene PCR Master Mix, respectively.

Therefore, a typical experiment consists of 4 wells needed for controls, plus 2 x (n) wells needed for the samples of interest, where (n) indicates the number of food samples of interest. Since a multiwell plate has 96 wells, 46 food samples can be analyzed during one PCR run if the GMO gene and the reference gene are analyzed in the same run.

Note: The procedure B is only applicable if the used real-time PCR instrument provides the opportunity to import external standard curves generated in a previous run.

2.4 Preparation of the PCR Mixes
Proceed as described below to prepare a 20 µl standard reaction. The PCR mixes for the GMO gene and the Reference gene must be set-up separately, using the respective Master Mixes.
Do not touch the upper surface of the PCR plate.

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, and 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.

Mix for the GMO Gene:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMO Gene Master Mix (vial 1, yellow cap)</td>
<td>13 µl</td>
</tr>
<tr>
<td>Enzyme Solution (vial 3, red cap)</td>
<td>1 µl</td>
</tr>
<tr>
<td>Dye Solution (vial 4, black cap)</td>
<td>1 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>15 µl</td>
</tr>
</tbody>
</table>

Mix for the Reference Gene:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Gene Master Mix (vial 2, green cap)</td>
<td>13 µl</td>
</tr>
<tr>
<td>Enzyme Solution (vial 3, red cap)</td>
<td>1 µl</td>
</tr>
<tr>
<td>Dye Solution (vial 4, black cap)</td>
<td>1 µl</td>
</tr>
<tr>
<td><strong>Total volume</strong></td>
<td>15 µl</td>
</tr>
</tbody>
</table>

3. Mix carefully but thoroughly by pipetting up and down. Do not vortex.
   • Pipet 15 µl PCR mix into each well or capillary.
   • For the samples of interest, add up to 5 µl sample DNA (if less than 5 µl add H₂O to 5 µl) to a well or capillary.
   • For the negative control, add 5 µl H₂O, PCR-grade (vial 7, colorless cap).
   • Procedure A: For the included (in-run) relative standard curves, add 5 µl of each dilution of Calibrator DNA (vial 5, purple cap) to the wells or capillaries.
   • Procedure B: For the positive control, add 5 µl Calibrator DNA (vial 5, purple cap) to a well or capillary.

4. Seal the plate or capillary accurately with an optical sealing foil or a stopper.

5. Place the plate in a swing bucket centrifuge and centrifuge at 1,500 x g for 30 s
   or
   Place the adapters, containing the capillaries, into a standard benchtop microcentrifuge.
   Note: Place the centrifuge adapters in a balanced arrangement within the centrifuge.
   Centrifuge at 700 x g for 5 s (3000 rpm in a standard benchtop microcentrifuge).

6. Cycle the samples as described above.
2.5 Calculation of Relative Amount of genetically modified maize

Procedure A – Quantification using included (in-run) relative standard curves

The use of calibration curves results in two values for every sample analyzed, (i.e., one for the GMO gene and one for the reference gene).

Note: Since the calibration curves are specific for the GMO and reference PCR, respectively, it is important that the generated values for the GMO and reference PCR are distinguishable. The percentage of Bt176 maize relative to the total maize content within the sample of interest must be calculated manually, with a spreadsheet program or with the analysis software of the used real-time PCR instrument (e.g. LightCycler® 480 instrument, Mx3005p system). Please refer to the manual of the used real-time PCR instrument for more detailed information. The calculation of the relative GMO content is based upon the resulting crossing points or Ct-values of one particular sample and the efficiency of the PCR. In brief, the crossing point or Ct-value is the cycle at which PCR amplification begins its exponential phase and is considered the point that is most reliably proportional to the logarithm of the initial concentration. The efficiency of the PCR describes the kinetics during the reaction. The overall reaction efficiency is represented by the slope of the calibration curve. Since primers and hydrolysis probes (5'-nuclease probes) for both parameter-specific components (GMO and reference gene) have individual PCR efficiencies, a calibration curve for each gene must be generated.

Notes:
• Quantify two independent sample preparations of each food sample and take the mean value as the final result.
• The ratio of GMO:reference in the Calibrator DNA provided with the kit is 1.0.
• Due to statistical reasons, the Bt176 maize detection and quantification becomes less reliable at low copy numbers, (i.e., results obtained from sample material with crossing points or Ct-values greater than 38 for either the GMO and/or the reference gene). Crossing points or Ct-values greater than 29 in the reference PCR indicate there is not enough maize DNA in the sample to reliably quantify 1% GMO content.
• DNA degradation during food processing may affect GMO quantification.

Procedure B – Quantification using external (imported) relative standard curves

For same real time PCR instruments it is possible to import external relative standard curves from a previous generated PCR run with the same instrument (e.g. LightCycler® 480 instrument or LightCycler® 2.0 instrument)

2.6 Related Procedures

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 of 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 genomic DNA from food or plant material) does not contain uracil and is therefore not degraded by this procedure. Since dTTP is replaced with dUTP and UNG is included in the foodproof GMO Bt176 Maize Quantification Kit, decontamination can be achieved with the provided reagents.
## 3. Appendix

### 3.1 Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Reason</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal increase is observed, even with positive controls.</td>
<td>Incorrect detection channel has been chosen.</td>
<td>Set Channel settings to FAM.</td>
</tr>
<tr>
<td>Pipetting errors or omitted reagents.</td>
<td>• Check for correct pipetting scheme and reaction setup. Repeat the PCR run. • Always run a positive control along with your samples.</td>
<td></td>
</tr>
<tr>
<td>No data acquisition programmed.</td>
<td>• Check the cycle programs. • Select acquisition mode “single” at the end of each annealing segment of the PCR program.</td>
<td></td>
</tr>
<tr>
<td>Inhibitory effects of the sample material (e.g., caused by insufficient purification).</td>
<td>• Use the recommended DNA sample preparation kit to purify template DNA. • Dilute samples or pipet a lower amount of sample DNA (e.g., 2.5 µl instead of 5 µl).</td>
<td></td>
</tr>
<tr>
<td>Fluorescence intensity is too low.</td>
<td>Inappropriate storage of kit components.</td>
<td>• Store the GMO Gene Master Mix, (vial 1, yellow cap) and the Reference Gene Master Mix, (vial 2, green cap) as indicated in Kit Contents Table, protect from light. • Avoid repeated freezing and thawing.</td>
</tr>
<tr>
<td>GMO Gene Master Mix or Reference Gene Master Mix is not homogeneously mixed.</td>
<td>Mix the GMO Gene Master Mix, (vial 1, yellow cap) and the Reference Gene Master Mix, (vial 2, green cap) thoroughly before pipetting.</td>
<td></td>
</tr>
<tr>
<td>Low initial amount of target DNA.</td>
<td></td>
<td>Increase the amount of sample DNA. Depending on the chosen DNA isolation method, inhibitory effects may occur.</td>
</tr>
<tr>
<td>Negative control samples are positive.</td>
<td>Carry-over contamination.</td>
<td>• 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.</td>
</tr>
<tr>
<td>Fluorescence intensity varies.</td>
<td>Insufficient centrifugation of the plate.</td>
<td>Always centrifuge the plate as described.</td>
</tr>
<tr>
<td>Surface of the sealing foil is dirty (e.g., by direct skin contact).</td>
<td></td>
<td>Always wear gloves when handling the plate.</td>
</tr>
</tbody>
</table>

### 3.2 References

4. Supplementary Information

4.1 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.

4.2 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.

4.3 Trademarks
foodproof® is a trademark of BIOTECON Diagnostics GmbH.
Other brand or product names are trademarks of their respective holders.

4.4 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.

5. Change Index
Version 1
First version of the package insert.

Version 2
License Notice changed.