December 2017

mericon® Quant GMO Handbook

For detection and quantification of genetically modified organisms in food or animal feed samples using real-time PCR

Sample to Insight

QIAGEN

Contents

Kit Contents

mericon **Quant GMO Detection Assays**		
See Table 1 for specific kits		
Number of reactions		**48**
Yellow	PCR Assay GMO*	4 x 12 reactions
Orange	PCR Assay Reference System*	4 x 12 reactions
Red	Quant Control DNA†	12 reactions
Green	Standard DNA	48 reactions
Blue	Multiplex PCR Master Mix‡	1040 µl
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml
	RNase-Free Water	1.9 ml
	50x ROX Dye Solution	45 µl
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* Contains target-specific primers and probes, as well as the internal control (IC).

† Quantification Control DNA: Contains DNA of the target GMO and reference species in a defined ratio.

‡ Contains HotStarTaq® *Plus* DNA Polymerase, dedicated multiplex real-time PCR buffer and dNTP mix (dATP, dCTP, dGTP, dTTP).

Table 1. *mericon* **Quant GMO Detection Assays**

Product name	**Catalog no.**
mericon Quant RR Soy (48) For specific detection and quantification of DNA from Roundup Ready soy	291514
mericon Quant MON 810 (48) For specific detection and quantification of DNA from MON 810 corn	291524

For more detailed information, see “Assay-specific information”, page 8.

Storage

mericon Quant GMO Detection Assays are shipped on dry ice. Multiplex PCR Master Mix and ROX dye should be stored at –30 to –15°C immediately upon receipt, in a constant-

temperature freezer. All remaining not reconstituted kit components should be stored at 2–8°C and protected from light. Stored under these conditions and handled correctly, assay performance remains unaffected until the date of expiration printed on the quality control label inside the kit box or envelope.

Reconstituted reagents of *mericon* Quant GMO Detection Assays should be dispensed into aliquots to avoid more than 5 freeze–thaw cycles and stored at 2–8°C for short-term storage (1 month) or –30 to –15°C for long-term storage.

Product Use Limitations

mericon Quant GMO Detection Assays are intended for molecular biology applications in food, animal feed and pharmaceutical product testing. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *mericon* Quant GMO Detection Assays is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Introduction

mericon Quant GMO Detection Assays are a ready-to-use system for the detection and quantification of specific DNA constructs from genetically modified organisms (GMO) in food, animal feed and pharmaceutical products using real-time polymerase chain reaction (PCR). These assays perform optimally on the Rotor-Gene® Q but have also been validated for block thermal cyclers. The Multiplex PCR Master Mix included in each kit contains QIAGEN proprietary technology including HotStarTaq *Plus* DNA Polymerase, patented multiplex PCR technology such as Factor MP and fast cycling technology including Q-Bond®. Multiplex PCR Master Mix is also highly tolerant to PCR inhibitors.

Each *mericon* Quant Assay contains an optimized mixture of PCR primer sets for a GMO-specific and a species-specific (Reference System) target sequence, each with an internal control (IC), plus probes labeled with distinct fluorescent dyes. In both systems, the test sample is detected with FAM™ reporter (495/520 nm), and the internal control is detected with MAX™ NHS Ester reporter (MAX; 524/557 nm). In addition, each kit includes a Quantification Control DNA, a Standard DNA, and all the reagents necessary to perform the analysis.

Principle

Detection of genetically modified organisms (GMO) by PCR is based on the amplification of a genetic construct used in GMOs. In real-time PCR, the amplified product is detected via target-specific fluorescent probes that bind to the amplified product. Accumulation of PCR product results in an increased fluorescent signal from the bound probes. Monitoring the fluorescence intensities during the PCR run (i.e., in real time) allows the detection of the accumulating PCR product without having to re-open the reaction tubes afterward.

The probes of *mericon* PCR Assays are sequence-specific oligonucleotides with a fluorophore and a quencher moiety attached. The fluorophore is at the 5' end of the probe, and the

quencher moiety is located at the 3' end. If the target DNA sequence is present, the probe is cleaved by the 5'–>3' exonuclease activity of HotStarTaq *Plus* DNA Polymerase during the extension phase of PCR. This separates the fluorophore and the quencher moiety resulting in a detectable fluorescence that is proportional to the amount of accumulated PCR product.

Quantification of the target GMO is carried out based on the alignment of the PCR results from a GMO-specific PCR system and a Reference PCR system. The GMO assay targets either a genetic construct commonly used in a desired GMO or a DNA sequence that is specific for a particular GMO (e.g., Roundup Ready soy). The Reference assay targets the plant species of the GMO (e.g., soy). Standard curves for both systems form the basis of the quantification experiment. They are generated with a defined dilution of the Standard DNA, which contains a defined ratio of GMO and Reference DNA.

The PCR primer sets for each *mericon* Quant GMO assay are highly specific. Targets are verified bioinformatically and experimentally. Cross-reactivity is tested with a continuously growing panel of selected targets for each *mericon* PCR Assay. Each assay can detect down to 10 target copies in a reaction.

Dedicated *mericon* sample preparation solutions are available from QIAGEN for a broad range of starting materials. These solutions were developed to complement *mericon* PCR Assays and provide a complete and efficient workflow for food safety testing.

HotStarTaq *Plus* DNA Polymerase

HotStarTaq *Plus* DNA Polymerase is a modified form of QIAGEN *Taq* DNA Polymerase. It is provided in an inactive state and has no enzymatic activity at ambient temperature. This prevents formation of misprimed products and primer–dimers during the reaction setup and first denaturation step. Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity and accurate quantification. The enzyme is first activated by a 5-minute, 95°C incubation step at the start of the reaction, which enables rapid and convenient setup at room temperature. In addition, the concentration of the polymerase in

the master mix is optimized to allow short extension times in the combined annealing/extension step of each PCR cycle.

Multiplex PCR Master Mix

The Multiplex PCR Master Mix is specifically developed for fast-cycling, multiplex, real-time PCR using sequence-specific probes. A novel additive in the buffer, Q-Bond, allows short cycling times on standard cyclers and on fast cyclers with rapid ramping rates. Q-Bond increases the affinity of HotStarTaq *Plus* DNA Polymerase for short single-stranded DNA, reducing the time required for primer/probe annealing to a few seconds. The buffer also contains Factor MP, which facilitates multiplex PCR. This synthetic factor increases the local concentration of primers and probes at the DNA template and stabilizes specifically bound primers and probes, allowing efficient annealing and extension. In addition, the Multiplex PCR Buffer is carefully formulated to be highly tolerant to inhibitors commonly present in food.

QuantiTect Nucleic Acid Dilution Buffer

QuantiTect Nucleic Acid Dilution Buffer is an optimized solution to dilute the nucleic acids used as the standards and the quantification or positive controls for *mericon* PCR Assays. The buffer stabilizes DNA standards and controls during dilution and reaction setup and prevents loss of nucleic acids on plastic surfaces, such as tubes or pipette tips. The buffer is ready to use and is free of DNases. Proper use of the buffer enables safe and accurate dilution of the small amounts of nucleic acids typically used as controls for analysis of nucleic acids. Aliquots of diluted standards and quantification or positive controls can be stored in QuantiTect Nucleic Acid Dilution Buffer at –30 to –15°C for up to 6 months. Repeated freezing and thawing should be avoided.

ROX Dye Solution, 50x

ROX Dye Solution is used with certain real-time cyclers to compensate for non-PCR-related variations in fluorescence detection. It is provided in a 50x solution for your convenience. It is not needed for Rotor-Gene Q, LightCycler® systems from Roche®, SmartCycler® instruments

from Cepheid, and Bio-Rad. It is necessary for most instruments from Applied Biosystems®, and is optional for Stratagene® cyclers from Agilent. Instructions for using the dye are provided in "Protocol: Detection and Quantification of GMO DNA by Real-Time PCR with ROX" on page 23.

Primer/probe mix with internal control

Each *mericon* PCR Assay includes rigorously designed primers and probes in a carefully balanced mix that amplify a target sequence and an internal control (IC) with high specificity. This internal control provides information regarding the presence of inhibitors in tested samples and the overall success of the PCR. MAX NHS Ester is employed as the reporter dye for the internal control. With excitation/emission maxima of *524/557* nm and a non-fluorescent quencher (Iowa Black®), MAX dye has a spectral profile comparable to HEX, JOE™, or VIC®, and can be used with most real-time cyclers.

Assay-specific information

mericon Quant RR Soy Kit

The *mericon* Quant RR Soy Kit is designed for the specific detection and quantification of Roundup Ready soy DNA in food, animal feed, and pharmaceutical products.

Limit of detection of the GMO and Reference System assays

The GMO-specific RR Soy Assay and the Reference System Assay (soy assay) of the *mericon* Quant RR Soy Kit can both detect down to 10 copies of target DNA in a reaction.

Specificity of the GMO and Reference System assays

The GMO-specific RR Soy Assay exhibits high specificity for Roundup Ready soy. No cross-reactivity was observed with other plant or animal species, GMOs, or genetic constructs not found in Roundup Ready soy (Table 2), using 50 ng of tested DNA.

Table 2. Results of cross-reactivity experiments for the RR Soy Assay

Genetic construct, GMO, or species	Result	Genetic construct, GMO, or species	Result
Roundup Ready soy	+	Bt11 corn	–
Bt176 corn	–	Lambda DNA	–
StarLink corn	–	LL rape	–
CaMV	–	GA21 corn	–
Roundup Ready canola	–	T25 corn	–
MON 810 corn	–	NK603 corn	–
Potato	–	Rice	–
Pig	–	Rye	–
Wheat	–	Barley	–
Cattle	–	Sheep	–

Cross-reactivity experiments are ongoing. For up-to-date information, visit **www.qiagen.com/mericonQuantGMO**.

The Reference System Assay (soy assay) exhibits high specificity for soy and Roundup Ready soy. No cross-reactivity was observed with other plant or animal species or GMOs (Table 3), using 50 ng of tested DNA.

Table 3. Results of cross-reactivity experiments for the Reference System Assay for the Quant RR Soy Kit

Genetic construct, GMO, or species	Result	Genetic construct, GMO, or species	Result
Soy	+	Roundup Ready soy	+
Potato	–	Cattle	–
Horse	–	Goat	–
Rye	–	Corn	–
Barley	–	Pig	–
Rice	–	Sheep	–
Canola	–	Wheat	–
Roundup Ready rice	–	CaMV	–
T25 corn	–	CGH351 corn	–
NK603 corn	–	Bt176 corn	–
Bt11 corn	–	MON 810 corn	–

Cross-reactivity experiments are ongoing. For up-to-date information, visit **www.qiagen.com/mericonQuantGMO**.

mericon Quant MON 810 Kit

The *mericon* Quant MON 810 Kit is designed for the detection and quantification of MON 810 corn in food, animal feed and pharmaceutical products.

Limit of detection of the GMO and Reference System assays

The GMO-specific MON 810 Assay and the Reference System Assay (corn assay) of the *mericon* Quant MON 810 Kit can both detect down to 10 copies of target DNA in a reaction.

Specificity of the GMO and Reference System assays

The GMO-specific MON 810 Assay exhibits high specificity for MON 810 corn. No cross-reactivity was observed with other plant or animal species, GMOs, or genetic constructs not found in MON 810 corn (Table 4), using 50 ng of tested DNA.

Table 4. Results from cross-reactivity experiments for the MON 810 Assay

Genetic construct, GMO, or species	Result	Genetic construct, GMO, or species	Result
MON 810 corn	+	356043 soy	–
Bt176 corn	–	EH92-527 potato	–
Bt11 corn	–	H7-1 sugar beet	–
MIR604 corn	–	Soy	–
3272 corn	–	Barley	–
NK603 corn	–	Potato	–
TC1507 corn	–	Rice	–
MON88017 corn	–	Wheat	–
MON863 corn	–	Rye	–
59122 corn	–	Cattle	–
98140 corn	–	Pig	–
Roundup Ready soy	–	Sheep	–
305423 soy	–	Goat	–

Cross-reactivity experiments are ongoing. For up-to-date information, visit **www.qiagen.com/mericonQuantGMO**.

The Reference System Assay (corn Assay) exhibits high specificity for corn and MON 810 corn. No cross-reactivity was observed with other plant or animal species or GMOs (Table 5), using 50 ng of tested DNA.

Table 5. Results from cross-reactivity experiments for the Reference System Assay for the Quant MON 810 Kit

Genetic construct, GMO, or species	Result	Genetic construct, GMO, or species	Result
Corn	+	Sheep	–
Soy	–	Goat	–
Canola	–	Horse	–
Rice	–	Hazelnut	–
Potato	–	Pecan nut	–
Rye	–	Macadamia	–
Wheat	–	Almond	–
Barley	–	Celery	–
Cattle	–	Mustard	–
Pig	–	Lupine	–

Cross-reactivity experiments are ongoing. For up-to-date information, visit **www.qiagen.com/mericonQuantGMO**.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment

- Rotor-Gene Q or other real-time PCR instrument* with fluorescence detection for approximately 520 nm (FAM fluorescence) and approximately 560 nm (for yellow dyes).
- For Rotor-Gene Q: Loading Block 72 x 0.1 ml Tubes, cat. no. 9018901, or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905
- Tube rack
- Microcentrifuge*
- Vortexer*

Material

- Pipettes (adjustable)*
- Sterile pipette tips with filters
- PCR plastics for the thermal cycler to be used
  - For Rotor-Gene Q: Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (cat. no. 981103 or 981106) or PCR Tubes, 0.2 ml, for use with 36 well rotor (cat. no. 981005 or 981008)

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. Use of yellow dye to detect the internal control of the *mericon* PCR Assays requires calibration on some instruments.

Reagents

- Nucleic acid isolation kit. We recommend the DNeasy® *mericon* Food Kit (see Ordering Information)
- Ice bucket with ice or cooling block

Important Notes

General precautions

The user should always pay attention to the following:

- Use gloves as well as sterile pipette tips with filters.
- All materials and media possibly containing DNA of the tested GMO should be autoclaved for 20 minutes at 120°C.
- Store and extract positive materials (specimens, positive controls and amplicons) separately from all other reagents, and add them to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature (15–25°C) before starting an assay.
- When thawed, mix the components (by pipetting repeatedly up and down or by pulse vortexing), and centrifuge briefly.

Relevant assay controls

Internal control

Each vial of the GMO and Reference System PCR assays contains an internal control to monitor successful PCR reaction and to detect possible PCR inhibition.

Standard dilutions

A Standard DNA is provided with each *mericon* Quant GMO Kit to prepare standard curves for the GMO and Reference System. It contains DNA of the target GMO, as well as of the reference species. For the Quant RR Soy Kit, the GMO:Reference ratio of the Standard DNA is 1:1. For the Quant MON 810 Kit, the GMO:Reference ratio of the Standard DNA is

1:0.57. The Standard DNA is diluted in defined steps and dilutions measured in the relevant copy number range for the GMO and for the reference species with both assay systems. Standard dilutions are integrated into the PCR reaction setup (see Table 9, page 20, and Table 16, page 26).

Quantification Control

The Quantification Control serves as a positive control for the quantification experiment. It contains DNA of the target GMO, as well as of the reference species. In all *mericon* Quantification Controls, the GMO:Reference DNA ratio is 1 ± 0.4%. During a quantification experiment, verification of this ratio ensures preparation of accurate standard curves and correct performance of quantification calculations. The Quantification Control is integrated into the PCR reaction setup (see Table 9, page 20, and Table 16, page 26).

Negative PCR control

Negative controls should be included in each analysis run to check for possible contamination of the *mericon* Assays during reaction setup. Instead of adding sample DNA to a reaction vial containing Multiplex PCR Master Mix, add the same volume of QuantiTect Nucleic Acid Dilution Buffer or RNase-free water.

Internal control calibration for real-time cyclers

MAX NHS Ester (MAX) dye is used to detect the internal control of mericon PCR Assays. Table 6 lists common thermal cyclers with their calibration requirement and the detection channel or filter set for this dye. Refer to the manufacturer's manual of the thermal cycler to be used for detailed calibration instructions.

Table 6. Calibration requirements and detection channel for MAX NHS Ester (MAX) dye

Thermal cycler	Dye calibration*	Filter suitable for MAX NHS Ester detection
Rotor-Gene Q Rotor-Gene 6000	Not required	Yellow
Applied Biosystems models 7000, 7300, 7500, 7700, 7900HT, StepOne™, StepOnePlus™	Required for new instruments†	VIC
Stratagene (Agilent) models Mx3005P®, Mx3000P®	Not required	Filter set 535/550 nm (HEX, JOE, VIC)

* For information on detection channel settings for instruments not listed in Table 6, contact QIAGEN Technical Services.

† If the instrument is new, a dye calibration for the individual channels (e.g., VIC) of the real-time cycler must be performed. See the manufacturer's manual for details on calibration.

Protocol: Detection and Quantification of GMO DNA by Real-Time PCR without ROX

Important points before starting

- Before beginning the procedure, read "Important Notes", page 15.
- Take time to familiarize yourself with the Rotor-Gene Q or other real-time PCR instrument to be used before starting the protocol. See the instrument user manual.

Things to do before starting

- Prepare the *mericon* PCR Assay GMO (tube with yellow lid).
  - Add 130 µl Multiplex PCR Master Mix (tube with blue lid) to each vial of *mericon* PCR Assay GMO (yellow lid). Mix by pipetting up and down 5 times or vortexing, and centrifuge briefly.
    Note: If the reconstituted PCR Assay GMO will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.
- Prepare the *mericon* PCR Assay Reference System (tube with orange lid)
  - Add 130 µl Multiplex PCR Master Mix (tube with blue lid) to each vial of *mericon* PCR Assay Reference System (orange lid). Mix by pipetting up and down 5 times or vortexing, and centrifuge briefly.
    Note: If the reconstituted PCR Assay Reference System will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.
- Dissolve the dried Quantification Control DNA (tube with red lid).
  - Add 150 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly.
    Note: If the dissolved Quantification Control will not be used entirely in one assay

run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

- Dissolve the dried Standard DNA (tube with green lid). Add 120 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial (Standard 1) and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly.
  - For this solution, use the preparation of defined Standard DNA dilutions. Assign the copy numbers given in Table 7 for the Quant RR Soy Kit and Table 8 for the Quant MON 810 Kit to the standard dilutions.

Table 7. Preparation of the Standard DNA dilution series for the Quant RR Soy Kit

Standard	Dilutions	Copy number per RRS PCR reaction	Copy number per Reference PCR reaction
Standard 1	Standard DNA dissolved in 120 µl QT NA Dilution Buffer	–	81,920
Standard 2	10 µl Standard 1 + 70 µl QT NA Dilution Buffer	10,240	10,240
Standard 3	10 µl Standard 2 + 70 µl QT NA Dilution Buffer	1280	1280
Standard 4	10 µl Standard 3 + 70 µl QT NA Dilution Buffer	160	160
Standard 5	10 µl Standard 4 + 70 µl QT NA Dilution Buffer	20	–

Table 8. Preparation of the Standard DNA dilution series for the Quant MON 810 Kit

Standard	Dilutions	Copy number per MON 810 PCR reaction	Copy number per Reference PCR reaction
Standard 1	Standard DNA dissolved in 120 µl QT NA Dilution Buffer	–	46,694
Standard 2	10 µl Standard 1 + 70 µl QT NA Dilution Buffer	10,240	5,837
Standard 3	10 µl Standard 2 + 70 µl QT NA Dilution Buffer	1280	730
Standard 4	10 µl Standard 3 + 70 µl QT NA Dilution Buffer	160	91
Standard 5	10 µl Standard 4 + 70 µl QT NA Dilution Buffer	20	–

- **Note**: If the reconstituted Standard DNA (Standard 1) will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –20°C for long-term storage.

- Before each use, all reagents need to be thawed completely, mixed (by repeated pipetting up and down or quick vortexing), and centrifuged briefly.

PCR reaction assignment

Assign Standard, Quantification Control and sample reactions to the respective assay systems as shown in Table 9. Standard dilutions, as well as Quantification Control and negative template controls (NTC), are assayed in duplicates. Sample extraction should be carried out at least twice and extracted DNA used in PCR reactions with both assay systems at 2 different concentrations (e.g., undiluted and 1:4 diluted).

Table 9. Assignment of Standard, Quantification Control and sample reactions

Component	Number of GMO PCR replicates	Number of Reference System PCR replicates
Standard 1	–	2
Standard 2	2	2
Standard 3	2	2
Standard 4	2	2
Standard 5	2	–
Quantification Control DNA	2	2
NTC	2	2
Sample extraction 1, dilution 1	1	1
Sample extraction 1, dilution 2	1	1
Sample extraction 2, dilution 1	1	1
Sample extraction 2, dilution 2	1	1

Procedure

1. Set up the Standard, Quantification Control and sample reactions according to Table 10. Keep all samples and reaction tubes on ice during setup.

   If using the Rotor-Gene Q, place the desired number of PCR tubes or strips into the adapters of the cooling block for the Rotor-Gene Q.

Table 10. Setup of Standard, Quantification Control and sample reactions for the GMO and Reference System *mericon* PCR Assays

Component	Standard dilutions	Quantification Control	Samples	Negative Control
Reconstituted *mericon* Assay (GMO or Reference System)	10 µl	10 µl	10 µl	10 µl
Respective Standard dilution	10 µl	–	–	–
Quantification Control DNA	–	10 µl	–	–
Sample DNA	–	–	10 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	–	10 µl
Total volume	**20 µl**	**20 µl**	**20 µl**	**20 µl**

2. Close the PCR tubes or strips and place them in the reaction chamber of the thermal cycler, securing them according to the instrument manual.

   If using the Rotor-Gene Q, make sure that the locking ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.

3. Program the thermal cycler. If using the Rotor-Gene Q or Rotor-Gene 6000, use the cycling protocol in Table 11 (page 22). For all other real-time cyclers, use the cycling protocol in Table 12 (page 22).

   Note: For information on instrument detection settings for the MAX NHS Ester dye (MAX) used to detect the internal control of *mericon* Assays, see Table 6 on page 17.

4. For the Rotor-Gene Q or Rotor-Gene 6000 make sure that "Perform Optimisation Before 1st Acquisition" in the "Gain Optimisation" menu is activated.

5. Start the PCR run.
6. Proceed to “Protocol: Analyzing the Results” on page 29.

Table 11. Cycling protocol for Rotor-Gene Q or Rotor-Gene 6000

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA Polymerase
3-step cycling:			
Denaturation	15 s	95°C	Data collection at 60°C
Annealing	15 s	60°C	
Extension	10 s	72°C	
Number of cycles	45		
Detection	**Reporter**	**Excitation/emission**	**Channel**
Target	FAM	495/520 nm	Green
Internal control	MAX	524/557 nm	Yellow

Table 12. Cycling protocol for real-time cyclers other than Rotor-Gene Q or Rotor-Gene 6000

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA Polymerase
3-step cycling:			
Denaturation	15 s	95°C	Data collection at 60°C
Annealing	23 s*	60°C	
Extension	10 s	72°C	
Number of cycles	45		
Detection	**Reporter**	**Excitation/emission**	**Channel**
Target	FAM	495/520 nm	Green (FAM)
Internal control	MAX	524/557 nm	Yellow (VIC)†

* For some instruments, the shortest annealing time possible is longer than 23 s (in the range of 32 s). Use the shortest annealing time permitted by the instrument.

† See Table 6 (page 17) for information on instrument-specific detection channel or filter set.

Protocol: Detection and Quantification of GMO DNA by Real-Time PCR with ROX

For certain real-time thermal cyclers, the use of a ROX passive reference dye during the PCR is necessary to compensate for variations in the fluorescence signal that are not related to the PCR.

Important points before starting

- Before beginning the procedure, read "Important Notes", page 15.
- Take time to familiarize yourself with the real-time PCR instrument to be used before starting the protocol. See the instrument user manual.

Things to do before starting

- Prepare the PCR Assay GMO (tube with yellow lid) and the PCR Assay Reference System (tube with orange lid) by adding Multiplex PCR Master Mix (tube with blue lid) and ROX dye to the tubes of both Assay systems according to Table 13. Mix by pipetting up and down 5 times or vortexing, and centrifuge briefly.

Table 13. Components to add to the *mericon* Assay

Thermal cycler	ROX dye	Multiplex PCR Master Mix
Applied Biosystems models 7000, 7300, 7700, 7900HT, StepOne, StepOnePlus	10.4 µl per vial	130 µl per vial
Applied Biosystems model 7500	5.2 µl per vial	130 µl per vial
Rotor-Gene models, Stratagene Mx models, LightCycler 480, SmartCycler models, Bio-Rad instruments	ROX reference dye not necessary	

Note: If the reconstituted *mericon* Assays will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –30 to –15°C for long-term storage.

- Dissolve the dried Quantification Control DNA (tube with red lid). Add 150 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial, and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly.

  Note: If the dissolved Quantification Control will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –30 to –15°C for long-term storage.

- Dissolve the dried Standard DNA (tube with green lid). Add 120 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial (Standard 1), and mix by pipetting up and down 5 times or vortexing. Centrifuge briefly.
  Use this solution for the preparation of defined Standard DNA dilutions. Assign the copy numbers given in Table 14 for the Quant RR Soy Kit and Table 15 (page 25) for the Quant MON 810 Kit to the standard dilutions.

Table 14. Preparation of the Standard DNA dilution series for the Quant RR Soy Kit

Standard	Dilutions	Copy number per RRS PCR reaction	Copy number per Reference PCR reaction
Standard 1	Standard DNA dissolved in 120 µl QT NA Dilution Buffer	–	81,920
Standard 2	10 µl Standard 1 + 70 µl QT NA Dilution Buffer	10,240	10,240
Standard 3	10 µl Standard 2 + 70 µl QT NA Dilution Buffer	1280	1280
Standard 4	10 µl Standard 3 + 70 µl QT NA Dilution Buffer	160	160
Standard 5	10 µl Standard 4 + 70 µl QT NA Dilution Buffer	20	–

Table 15. Preparation of the Standard DNA dilution series for the Quant MON 810 Kit

Standard	Dilutions	Copy number per MON 810 PCR reaction	Copy number per Reference PCR reaction
Standard 1	Standard DNA dissolved in 120 µl QT NA Dilution Buffer	–	46,694
Standard 2	10 µl Standard 1 + 70 µl QT NA Dilution Buffer	10,240	5,837
Standard 3	10 µl Standard 2 + 70 µl QT NA Dilution Buffer	1280	730
Standard 4	10 µl Standard 3 + 70 µl QT NA Dilution Buffer	160	91
Standard 5	10 µl Standard 4 + 70 µl QT NA Dilution Buffer	20	–

Note: If the reconstituted Standard DNA (Standard 1) will not be used entirely in one assay run, make appropriate aliquots to avoid more than 5 freeze–thaw cycles, and store the aliquots at 2–8°C for short-term storage (1 month) or –30 to –15°C for long-term storage.

- Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

PCR reaction assignment

Assign the Standard, Quantification Control and sample reactions to the respective assay systems as shown in Table 16 (page 26). Standard dilutions, as well as Quantification Control and negative template controls (NTC), are assayed in duplicates. Sample extraction should be carried out at least twice and the extracted DNA used in PCR reactions with both assay systems at 2 different concentrations (e.g., undiluted and 1:4 diluted).

Table 16. Assignment of Standard, Quantification Control and sample reactions

Component	Number of GMO PCR replicates	Number of Reference System PCR replicates
Standard 1	–	2
Standard 2	2	2
Standard 3	2	2
Standard 4	2	2
Standard 5	2	–
Quantification Control DNA	2	2
NTC	2	2
Sample extraction 1, dilution 1	1	1
Sample extraction 1, dilution 2	1	1
Sample extraction 2, dilution 1	1	1
Sample extraction 2, dilution 2	1	1

Procedure

1. Set up the Standard, Quantification Control and sample reactions according to Table 17 or Table 18 (page 27), depending on the thermal cycler to be used. Keep all samples and reaction tubes on ice during setup.

Table 17. Setup of Standard, Quantification Control and sample reactions for Applied Biosystems models 7000, 7300, 7700, 7900HT, StepOne and StepOnePlus

Component	Standard dilutions	Quantification Control	Samples	Negative Control
Reconstituted *mericon* Assay (GMO or Reference System)	10.8 µl	10.8 µl	10.8 µl	10.8 µl
Respective Standard dilution	9.2 µl	–	–	–
Quantification Control DNA	–	9.2 µl	–	–
Sample DNA	–	–	9.2 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	–	9.2 µl
Total volume	**20 µl**	**20 µl**	**20 µl**	**20 µl**

Table 18. Setup of Standard, Quantification Control and sample reactions for Applied Biosystems model 7500

Component	Standard dilutions	Quantification Control	Samples	Negative Control
Reconstituted *mericon* Assay (GMO or Reference System)	10.4 µl	10.4 µl	10.4 µl	10.4 µl
Respective Standard dilution	9.6 µl	–	–	–
Quantification Control DNA	–	9.6 µl	–	–
Sample DNA	–	–	9.6 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	–	9.6 µl
Total volume	**20 µl**	**20 µl**	**20 µl**	**20 µl**

2. Close the PCR tubes or strips, and place them in the reaction chamber of the thermal cycler, securing them according to the instrument manual.
3. Program the real-time cycler according to Table 19.

   Note: For information on instrument detection settings for the MAX NHS Ester dye (MAX) used to detect the internal control of *mericon* Assays, see Table 6 on page 17, and start the PCR run.

Table 19. Cycling protocol for real-time cyclers using ROX reference dye

Step	Time	Temperature	Comments
Initial PCR activation step	5 min	95°C	Activation of HotStarTaq *Plus* DNA Polymerase
3-step cycling:			
Denaturation	15 s	95°C	Data collection at 60°C
Annealing	23 s*	60°C	
Extension	10 s	72°C	
Number of cycles	45		

Detection	Reporter	Excitation/emission	Channel
Target	FAM	520 nm	Green (FAM)
Internal control	MAX	520 nm	Yellow (VIC)†

* For some instruments, the shortest annealing time possible is longer than 23 s (in the range of 32 s). Use the shortest annealing time permitted by the instrument.

† See Table 6 (page 17) for information on instrument-specific detection channel or filter set.

4. Start the PCR run.
5. Proceed to “Protocol: Analyzing the Results” on page 29.

Protocol: Analyzing the Results

Qualitative GMO detection

Qualitative determination of the presence or absence of DNA from a genetically modified organism (GMO) is carried out based on the amplification of the target sequence by the GMO-specific *mericon* PCR assay. Amplification is visualized in real time on the amplification plot generated by the application software of the real-time PCR instrument used. A positive result is visible as a final point on the fluorescence curve that lies clearly above the threshold. Figures 1–3 are examples of possible outcomes, which are summarized in Table 20 (page 30).

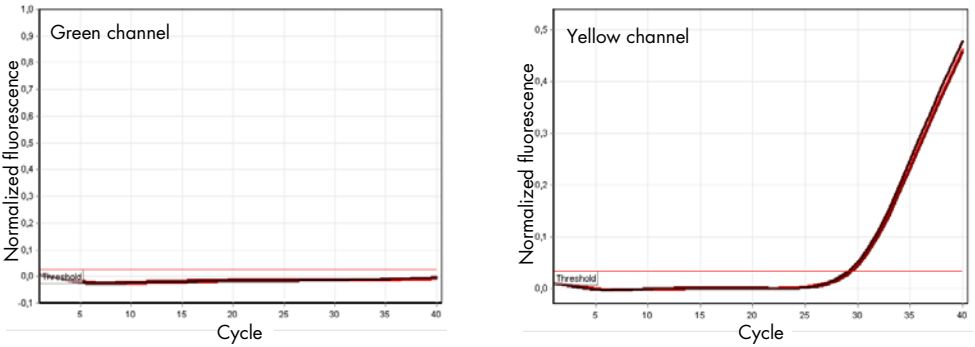

Figure 1. The sample is negative for the tested GMO or genetic construct. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

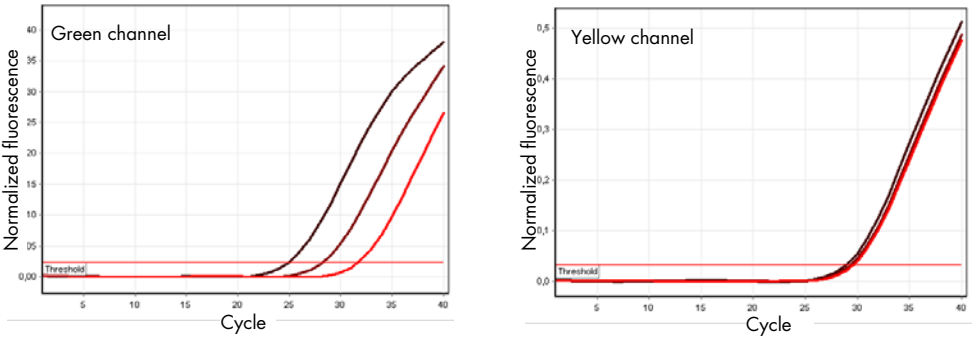

Figure 2. The sample is positive for the tested GMO or genetic construct. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of target DNA in the sample and a successful PCR.

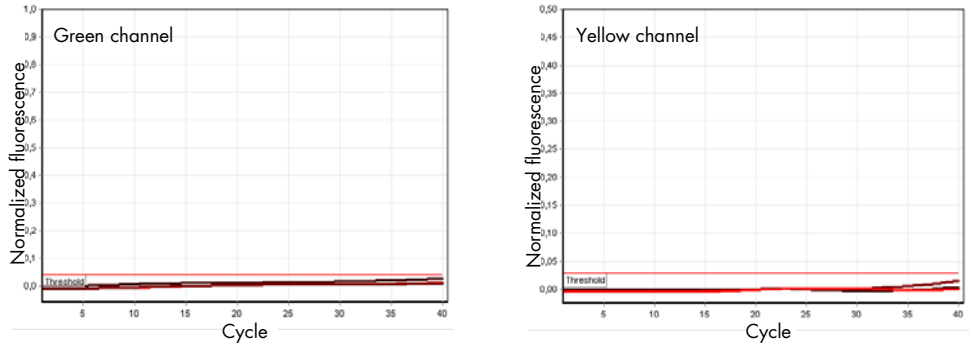

Figure 3. The PCR is inhibited. No amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 20. Summary of possible outcomes

Amplification of internal control	Amplification of sample	Result
+	+	Sample is positive
+	–	Sample is negative
–	–	PCR failed (inhibition)

Partial inhibition of the PCR due to the presence of detectable but tolerable concentrations of inhibitors in the samples is typically indicated by a shift of the Internal Control to higher cycle values. As a guideline, the uninhibited Internal Control should give a stable cycle value between 28 and 32. A cycle value above 32 indicates inhibition.

In the event of PCR inhibition, dilute the extracted samples 1:10 with QuantiTect Nucleic Acid Dilution Buffer or RNase-free water and repeat the test.

If the DNA template concentration in the PCR reaction is very high, a shift of the Internal Control to lower cycle values might occur, which does not influence its sensitivity toward PCR inhibitors or amplification of the target DNA.

Quantitative GMO detection

Quantification of the genetically modified organism (GMO) is carried out based on the alignment of the PCR results from the GMO-specific PCR system and the Reference PCR system. Results are obtained according to the reaction scheme illustrated in Table 9, page 20, or Table 16, page 26.

Standard dilutions are measured in the relevant concentration range for detection of the GMO and overall plant species content of the sample. The GMO DNA normally represents only a small fraction of the overall sample DNA. It is measured at the lower end of the copy number range (Table 7 and Table 8, page 19, or Table 14 and Table 15, pages 24 and 25).

The plant species DNA normally represents the major part of the sample DNA. It is measured at the upper end of the copy number range (Table 7 and Table 8, page 19, or Table 14 and Table 15, pages 24 and 25).

Cycle values of the standard dilutions obtained with each assay system are plotted against the logarithm of the respective copy numbers to provide a separate standard curve for the

GMO system and the Reference system. Cycle values of the unknown samples are compared with both standard curves to determine the copy numbers of the GMO and Reference species in the unknown sample. From the ratio of the determined sample copy numbers for both systems (reference system set at 100%), the percentage of GMO in the unknown sample is calculated.

If possible, it is recommended to use the software of the applied real-time cycler for data analysis. From the acquired C_T values of the standard dilutions, the instrument normally generates standard curves and calculates slope, as well as goodness-of-fit of the linear regression. An accurate GMO quantification relies on the precision of the standard curves. Accuracy of the quantification experiment is monitored via the Quantification Control. The Quantification Control contains a defined GMO:Reference ratio of 1 ± 0.4%. Quantification results are representative if the GMO content of the Quantification Control is determined within a 0.6–1.4% range.

GMO quantification is carried out with DNA samples of two separate DNA extractions, applying two different DNA dilutions to verify reproducibility and consistency of the calculated GMO content. If the GMO content of both dilutions differs strongly, one or both assay systems are influenced by PCR inhibitors. In this case, the quantification experiment must be repeated with additional sample dilutions until the GMO contents align.

Table 21 (page 33) is an example of possible data obtained from a representative RR Soy quantification experiment.

Table 21. Example of possible data outcome and calculated GMO content from a representative RRS quantification experiment

Component	GMO	Copy number	Ref	Copy number	% GMO
Standard 1	–	–	18.86 18.55	81,920	–
Standard 2	23.35 23.29	10,420	21.83 21.74	10,420	–
Standard 3	25.78 26.10	1250	24.60 24.86	1250	–
Standard 4	29.30 29.22	160	27.87 27.71	160	–
Standard 5	32.90 32.92	20	–	–	–
R^2 value (linearity)	0.9942	–	0.9988	–	–
y-axis intercept	37.29	–	35.15	–	–
Slope standard curve	–3.55	–	–3.34	–	–
PCR efficiency	91%	–	99%	–	–
Quant. Control replicate 1	30.15	102	21.64	10,986	0.93%
Quant. Control replicate 2	29.89	121	21.56	11,608	1.04%
Extraction 1, undiluted	27.75	485	20.67	21,425	2.26%
Extraction 1, 1:4 diluted	29.64	143	22.51	6,035	2.37%
Extraction 2, undiluted	25.60	1,954	18.64	86,686	2.25%
Extraction 2, 1:4 diluted	27.00	789	20.02	33,518	2.35%

Troubleshooting Guide

The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
No signal with the Quantification Control		
a)	The selected fluorescence channel for PCR data analysis does not comply with the protocol	For data analysis, select the green channel (FAM) for the samples and the yellow channel (VIC or corresponding filter set) for the internal control. Please see the cycling protocols in Table 11 or Table 12 (page 22), or in Table 19 (page 27). Refer to the manufacturer's manual of the cycler to be used.
b)	Incorrect programming of the real-time PCR instrument	Compare the temperature profile with the protocol. See the cycling protocols in Table 11 or Table 12 (page 22), or in Table 19 (page 27). Refer to the manufacturer's manual of the cycler to be used.
c)	Incorrect configuration of the PCR	Check that reactions were set up according to Table 10 (page 21) or Table 17 or Table 18 (pages 26 and 27). Repeat the PCR, if necessary.
d)	Kit or kit components incorrectly stored	See "Storage" (page 3). Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
e)	The *mericon* PCR Assay has expired	Check the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Determination of a GMO content of the Quantification Control, which is not in the 1 ± 0.4% range		
a)	Standard curves are not prepared correctly	Make sure that the Standard DNA dilutions were strictly prepared according to Table 7 or Table 8 (page 19) or Table 14 or Table 15 (pages 24 and 25). Provided copy numbers refer to final copy numbers per PCR reaction.
b)	Standard curves are not plotted or read off properly	For preparation of the standard curves C_T values (y-axis) are plotted against the logarithm of the copy numbers (x-axis) provided in Table 7 or Table 8 (page 19) or Table 14 or Table 15 (pages 24 and 25). The data correlation needs to follow a linear regression. Copy numbers correlating with C_T values of the Quantification Control should be read off the standard curves by the real-time cycler software or alternatively calculated via the linear equation corresponding to the data fit.

		Comments and suggestions
c)	Quality of the standard curves is low	Accuracy of the standard curves is verified via the Quantification Control, which contains a GMO:Reference ratio of 1 ± 0.4%. Standard curves are correctly prepared if the Quantification Control is determined within a 0.6–1.4% range.
Deviation of determined GMO content of sample dilutions from one DNA extraction		
	Inhibition of one of both PCR systems	Repeat the quantification experiment with additional sample dilutions until the GMO contents align.
Weak or no signal in the internal amplification control		
a)	The PCR conditions do not comply with the protocol	Check that PCR conditions match the cycling protocols in Table 11 or Table 12 (page 22), or in Table 19 (page 27). Repeat the PCR with corrected settings, if necessary.
b)	The PCR was inhibited	Use the recommended DNA isolation method and closely follow the manufacturer's instructions. QIAGEN offers dedicated sample preparation kits developed to complement *mericon* PCR Assays, and provide a complete and efficient workflow for food safety testing.
c)	Kit or kit components incorrectly stored	See "Storage" (page 3). Check the storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
d)	The *mericon* PCR Assay has expired	Check the expiration date (see the kit label) of the reagents and use a new kit, if necessary.
Signals present for the negative controls		
a)	Contamination occurred during PCR setup	Repeat the PCR with new reagents in replicates. If possible, close the PCR tubes directly after addition of the sample to be tested. Make sure to pipet the positive controls last. Make sure that work space and instruments are decontaminated at regular intervals.
b)	Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

Ordering Information

Product	Contents	Cat. no.
mericon Quant RR Soy (48)	For 48 reactions: PCR Assay RR Soy, PCR Assay Reference System, Quant Control DNA, Standard DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291514
mericon Quant MON 810 (48)	For 48 reactions: PCR Assay MON 810, PCR Assay Reference System, Quant Control DNA, Standard DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291524
Related products		
mericon **GMO Assays**		
mericon Screen 35S Kit (24)*	For 24 reactions: PCR Assay Screen 35S, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291013
mericon Screen Nos Kit (24)*	For 24 reactions: PCR Assay Screen Nos, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291043

* Larger kit sizes available; please inquire.

mericon RR Soy (24)*	For 24 reactions: PCR Assay RR Soy, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291113
***mericon* Pathogen Detection Assays**		
mericon Salmonella spp Kit (24)*	For 24 reactions: PCR Assay Salmonella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290013
mericon L. monocytogenes Kit (24)*	For 24 reactions: PCR Assay L. monocytogenes, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290023
mericon Campylobacter spp Kit (24)*	For 24 reactions: PCR Assay Campylobacter spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290033
mericon Campylobacter triple Kit (24)*	For 24 reactions: PCR Assay Campylobacter triple, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290043
mericon VTEC stx1/2 Kit (24)*	For 24 reactions: PCR Assay VTEC stx1/2, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290053

* Larger kit sizes available; please inquire.

mericon Cronobacter spp Kit (24)*	For 24 reactions: PCR Assay Cronobacter spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290063
mericon S. aureus Kit (24)*	For 24 reactions: PCR Assay S. aureus, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290073
mericon Legionella spp Kit (24)*	For 24 reactions: PCR Assay Legionella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290083
mericon L. pneumophila Kit (24)*	For 24 reactions: PCR Assay L. pneumophila, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290093
mericon Shigella spp Kit (24)*	For 24 reactions: PCR Assay Shigella spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290103
mericon Y. enterocolitica Kit (24)*	For 24 reactions: PCR Assay Y. enterocolitica, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290113

* Larger kit sizes available; please inquire.

***mericon* Animal and Plant Identification Assays**		
mericon Pig Kit (24)*	For 24 reactions: PCR Assay Pig, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292013
mericon Soy Kit (24)*	For 24 reactions: PCR Assay Soy, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293013
***mericon* sample preparation kits**		
DNeasy *mericon* Food Kit (50)	50 QIAquick® Spin Columns, Proteinase K, buffers	69514
mericon DNA Bacteria Kit (100)	Fast Lysis Buffer	69525
mericon DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534
Instrumentation		
Rotor-Gene Q 5plex	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire

* Larger kit sizes available; please inquire.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Revision History

Document revision history	
R2 11/2017	Changed specifications for the GMO:Reference DNA ratio for the Quantification Control and cycle value indicative of inhibition (pages 16, 31, 32, 34, 35) Updated Safety Information (page 4)

Notes

Notes

Limited License Agreement for *mericon* Quant GMO Assays

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