February 2011

mericon™ GMO Detection Handbook

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

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Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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- microRNA research and RNAi
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Contents

Kit Contents

***mericon* GMO Detection Assays** See Table 1, page 5 for specific kits		**(24)**	**(96)**
Number of reactions		**24**	**96**
Yellow	*mericon* Assay*	2 x 12 reactions	1 x 96 reactions
Red	Positive Control DNA	20 reactions	20 reactions
	QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml	1.5 ml
	RNase-Free Water	1.9 ml	1.9 ml
Blue	Multiplex PCR Master Mix†	2 x 130 µl	1040 µl
	50x ROX Dye Solution	45 µl	45 µl
	Handbook	1	1

* Contains target-specific primers and probes, as well as the internal control (IC)

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

Table 1. *mericon* GMO Detection Assays

Product name	Catalog no. (24)	Catalog no. (96)
mericon Screen 35S Kit For general detection of GMOs with the 35S promoter of the cauliflower mosaic virus*	291013	291015
mericon Screen Nos Kit For general detection of GMOs with the nos terminator from *Agrobacterium tumefaciens**	291043	291045
mericon RR Soy Kit For specific detection of Roundup Ready soy*	291113	291115
mericon GMO Screen 35S-pat Kit For general detection of GMOs with the 35S-pat construct*	291023	291025
mericon GMO Screen CTP2-CP4EPSPS Kit For general detection of GMOs with the CTP2-CP4EPSPS construct*	291053	291055
mericon GMO Screen bar Kit For general detection of GMOs with the *bar* gene	291063	291065
mericon Mon 810 Corn Kit For specific detection of DNA from MON 810 corn*	291073	291075

* For more detailed information, see “Assay-specific information”, page 11.

Storage

mericon GMO Detection Assays are shipped on dry ice. Multiplex PCR Master Mix and ROX dye should be stored immediately at –20°C 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* 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 –20°C for long-term storage.

Product Use Limitations

mericon 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.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN® product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Quality Control

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

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding *mericon* GMO Detection Assays or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

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

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

mericon GMO Detection Assays are a ready-to-use system for the detection of specific DNA constructs of 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 been validated for block thermal cyclers as well. 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* Assay is an optimized mixture of PCR primer sets for a GMO-specific target sequence and an internal control (IC), plus probes labeled with two distinct fluorescent dyes (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 positive control DNA and all of the reagents necessary to perform the analysis.

Principle

Detection of genetically modified organisms (GMO) by the polymerase chain reaction (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 after the PCR run.

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.

The PCR primer set for each assay is highly specific and targets either a genetic construct commonly used in GMOs or a DNA sequence that is specific for a particular GMO. Targets are verified bioinformatically and experimentally. Cross-reactivity has been thoroughly tested with a 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 the formation of misprimed products and primer–dimers during reaction setup and the first denaturation step. Competition for reactants by PCR artifacts is therefore avoided, enabling high PCR specificity and accurate quantification. The enzyme is activated first at the start of a reaction by a 5-minute, 95°C incubation step, which enables reactions to be set up rapidly and conveniently 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 positive controls for *mericon* PCR Assays. The buffer stabilizes DNA controls during dilution and reaction setup and prevents loss of nucleic acids on plastic surfaces, such as tubes or pipet 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 positive control can be stored in QuantiTect Nucleic Acid Dilution Buffer at –15 to –30°C for up to 6 months. Repeated freezing and thawing should be avoided.

ROX Dye Solution, 50x

For certain real-time cyclers, the presence of ROX passive reference dye in real-time PCR compensates for non-PCR–related variations in fluorescence detection. The use of ROX dye is necessary for most instruments from Applied Biosystems® and is optional for Stratagene® cyclers from Agilent. ROX dye is not necessary for the Rotor-Gene Q, LightCycler® systems from Roche®, SmartCycler® instruments from Cepheid, and Bio-Rad instruments. ROX dye is provided in a 50x solution and instructions for using the dye are provided in the protocol "Detection of GMO DNA by real-time PCR with ROX" on page 25.

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 the 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 Screen 35S Kit

The *mericon* Screen 35S Kit is designed for the detection of the 35S promoter of the cauliflower mosaic virus in food, animal feed, and pharmaceutical products.

Limit of detection

The *mericon* Screen 35S Kit can detect down to 10 copies of target DNA in a reaction.

Specificity

The *mericon* Screen 35S Kit exhibits high specificity for the 35S promoter of the cauliflower mosaic virus. No cross-reactivity was observed with other genetic constructs or with plant or animal species not containing this genetic construct (Table 2), using 50 ng of the tested DNA.

Table 2. Results from cross-reactivity experiment*

Genetic construct, GMO, or species	Result	Genetic construct, GMO, or species	Result
35S promoter	+	MON 810 corn	+
CBH351 corn	+	Roundup Ready soy	+
Bt176 corn	+	T25 corn	+
Bt11 corn	+	LL canola	+
NK603 corn	+	Rye	–
Barley	–	Lambda DNA	–
Goat	–	GA21 corn	–
Potato	–	Cattle	–
Horse	–	Roundup Ready canola	–
Rice	–	Pig	–
Wheat	–	Sheep	–

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

mericon Screen Nos Kit

The *mericon* Screen Nos Kit is designed for the detection of the nos terminator from *Agrobacterium tumefaciens* in food, animal feed, and pharmaceutical products.

Limit of detection

The *mericon* Screen Nos Kit can detect down to 10 copies of target DNA in a reaction.

Specificity

The *mericon* Screen Nos Kit exhibits high specificity for the nos terminator from *Agrobacterium tumefaciens*. No cross-reactivity was observed with other genetic constructs or with plant or animal species not containing this genetic construct (Table 3), using 50 ng of the tested DNA.

Table 3. Results from cross-reactivity experiment*

Genetic construct, GMO, or species	Result	Genetic construct, GMO, or species	Result
Bt11 corn	+	Roundup Ready soy	+
CBH351 corn	+	Roundup Ready GA21 corn	+
NK603 corn	+	LL T25 corn	–
Barley	–	Rye	–
Goat	–	CaMV	–
MON 810 corn	–	Potato	–
Cattle	–	Horse	–
Lambda DNA	–	Roundup Ready canola	–
Rice	–	Pig	–
Bt176 corn	–	LL canola	–
Wheat	–	Sheep	–

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

mericon RR Soy Kit

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

Limit of detection

The *mericon* RR Soy Kit can detect down to 10 copies of target DNA in a reaction.

Specificity

The *mericon* RR Soy Kit 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 4), using 50 ng of the tested DNA.

Table 4. Results from cross-reactivity experiment*

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/mericonGMO.

mericon GMO Screen 35S-pat Kit

The *mericon* GMO Screen 35S-pat Kit is designed for the detection of the 35S-pat construct in food, animal feed, and pharmaceutical products. This construct links the *pat* gene from *Streptomyces viridochromogenes* to the 35S promoter of cauliflower mosaic virus. The promoter triggers expression of phosphinotricin-acetyltransferase conferring resistance to the herbicide phosphinotricin.

Limit of detection

The *mericon* GMO Screen 35S-pat Kit can detect down to 10 copies of target DNA in a reaction.

Specificity

The *mericon* GMO Screen 35S-pat Kit exhibits high specificity for the 35S-pat construct. No cross-reactivity was observed with other genetic constructs or with plant or animal species not containing this genetic construct (Table 5), using 50 ng of the tested DNA.

Table 5. Results from cross-reactivity experiment*

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

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

mericon GMO Screen CTP2-CP4EPSPS Kit

The *mericon* GMO Screen CTP2-CP4EPSPS Kit is designed for the detection of the CTP2-CP4EPSPS construct in food, animal feed, and pharmaceutical products. This construct links the *ctp2* gene of *Arabidopsis thaliana* to a glyophosphate-tolerant form of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) isolated from *Agrobacterium tumefaciens* strain CP4. As a result, GMOs with this construct are resistant to Roundup Ready.

Limit of detection

The *mericon* GMO Screen CTP2-CP4EPSPS Kit can detect down to 10 copies of target DNA in a reaction.

Specificity

The *mericon* GMO Screen CTP2-CP4EPSPS Kit exhibits high specificity for the construct CTP2-CP4EPSPS. No cross-reactivity was observed with other genetic constructs or with plant or animal species not containing this genetic construct (Table 6, page 16), using 50 ng of the tested DNA.

Table 6. Results from cross-reactivity experiment*

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

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

mericon GMO Screen bar Kit

The *mericon* GMO Screen bar Kit is designed for the detection of the *bar* gene in food, animal feed, and pharmaceutical products. Expression of the *bar* gene confers resistance to bialaphos, a broad-spectrum herbicide.

Limit of detection

The *mericon* GMO Screen bar Kit can detect down to 10 copies of target DNA in a reaction.

Specificity

The *mericon* GMO Screen bar Kit exhibits high specificity for the *bar* gene. No cross-reactivity was observed with other genetic constructs or with plant or animal species not containing this genetic construct (Table 7, page 17), using 50 ng of the tested DNA.

Table 7. Results from cross-reactivity experiment*

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

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

mericon Mon 810 Corn Kit

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

Limit of detection

The *mericon* Mon 810 Corn Kit can detect down to 10 copies of target DNA in a reaction.

Specificity

The *mericon* Mon 810 Corn Kit exhibits high specificity for MON 810 corn. No cross-reactivity was observed with other genetically modified organisms or with other plant or animal species (Table 8, page 18), using 50 ng of the tested DNA.

Table 8. Results from cross-reactivity experiment*

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

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

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 material safety data sheets (MSDSs), available from the product supplier.

- Nucleic acid isolation kit. We recommend the DNeasy® *mericon* Food Kit (see Ordering Information)
- Pipets (adjustable)*
- Sterile pipet tips with filters
- 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). See Table 9 on page 21 for examples.
- 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)†
- 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*
- Ice bucket with ice or cooling block

* 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. See Table 9, page 16 for details.

Important Notes

General precautions

The user should always pay attention to the following:

- Use gloves as well as sterile pipet tips with filters.
- All materials and media possibly containing DNA of the tested GMO should be autoclaved for 20 min 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 *mericon* Assay contains an internal control to detect possible PCR inhibition.

Negative PCR control

Negative controls should be included in each analysis run to check for possible contamination of the *mericon* Assay 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.

Positive PCR control

Positive controls should be included in each analysis run to check the functionality of the Multiplex PCR Master Mix. Instead of adding sample DNA to a reaction vial containing Multiplex PCR Master Mix, add the same volume of the positive control included in the kit.

Internal control calibration for real-time cyclers

MAX NHS Ester (MAX) dye is used to detect the internal control of *mericon* PCR Assays. Table 9 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 9. 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 9, 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 of GMO DNA by Real-Time PCR without ROX

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 20.
- 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.
- Make sure that at least one positive and one negative control are included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube with yellow lid).

  24-sample kit: Add 130 µl Multiplex PCR Master Mix (tube with blue lid) to each vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

  96-sample kit: Add 1040 µl Multiplex PCR Master Mix (tube with blue lid) to the vial of *mericon* Assay (yellow lid). Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

  Note: If the reconstituted *mericon* Assay 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 Positive Control DNA (tube with red lid). Add 200 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 positive 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.

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

Procedure

1. **Set up the sample and control reactions according to Table 10, page 23. 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 sample and control reactions

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted *mericon* Assay	10 µl	10 µl	10 µl
Sample DNA	10 µl	–	–
Dissolved Positive Control DNA	–	10 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	10 µl
Total volume	**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 24). For all other real-time cyclers, use the cycling protocol in Table 12 (page 24).**

   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 9 on page 21.

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 the 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 9, page 21 for information on instrument-specific detection channel or filter set.

Protocol: Detection 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 20.
- Take time to familiarize yourself with the real-time PCR instrument to be used before starting the protocol. See the instrument user manual.
- Make sure that at least one positive and one negative control are included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube with yellow lid) by adding Multiplex PCR Master Mix (tube with blue lid) and ROX dye to the tube(s) of *mericon* Assay 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	Kit size	ROX dye	Multiplex PCR Master Mix
Applied Biosystems models 7000, 7300, 7700, 7900HT, StepOne, StepOnePlus	24	10.4 µl per vial	130 µl per vial
	96	83.2 µl	1040 µl
Applied Biosystems model 7500	24	5.2 µl per vial	130 µl per vial
	96	41.6 µl	1040 µl
Rotor-Gene models, Stratagene Mx models, LightCycler 480, SmartCycler models, Bio-Rad instruments	ROX reference dye not necessary		

Note: If the reconstituted *mericon* Assay 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 Positive Control DNA (tube with red lid). Add 200 µl of QuantiTect Nucleic Acid Dilution Buffer to the vial and mix by pipetting up and down 5 times or vortexing.

  Note: If the dissolved positive 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.

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

Procedure

1. **Set up the sample and control reactions according to Table 14 or Table 15 (page 27), depending on the thermal cycler to be used. Keep all samples and reaction tubes on ice during setup.**

Table 14. Setup of sample and control reactions for Applied Biosystems models 7000, 7300, 7700, 7900HT, StepOne, and StepOnePlus

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted *mericon* Assay	10.8 µl	10.8 µl	10.8 µl
Sample DNA	9.2 µl	–	–
Dissolved Positive Control DNA	–	9.2 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	9.2 µl
Total volume	**20 µl**	**20 µl**	**20 µl**

Table 15. Setup of sample and control reactions for Applied Biosystems model 7500

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted *mericon* Assay	10.4 µl	10.4 µl	10.4 µl
Sample DNA	9.6 µl	–	–
Dissolved Positive Control DNA	–	9.6 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	9.6 µl
Total volume	**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 16 (page 28).**

   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 9 on page 21.
4. **Start the PCR run.**
5. **Proceed to the protocol "Analyzing the Results" on page 29.**

Table 16. 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	Emission maximum	Channel
Target	FAM	520 nm	Green (FAM)
Internal control	MAX	560 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 9, page 21 for information about instrument-specific detection channel or filter set.

Protocol: Analyzing the Results

Determining the presence or absence of DNA from genetically modified organisms (GMO) is carried out based on the amplification of the target sequence and 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 17 (page 30).

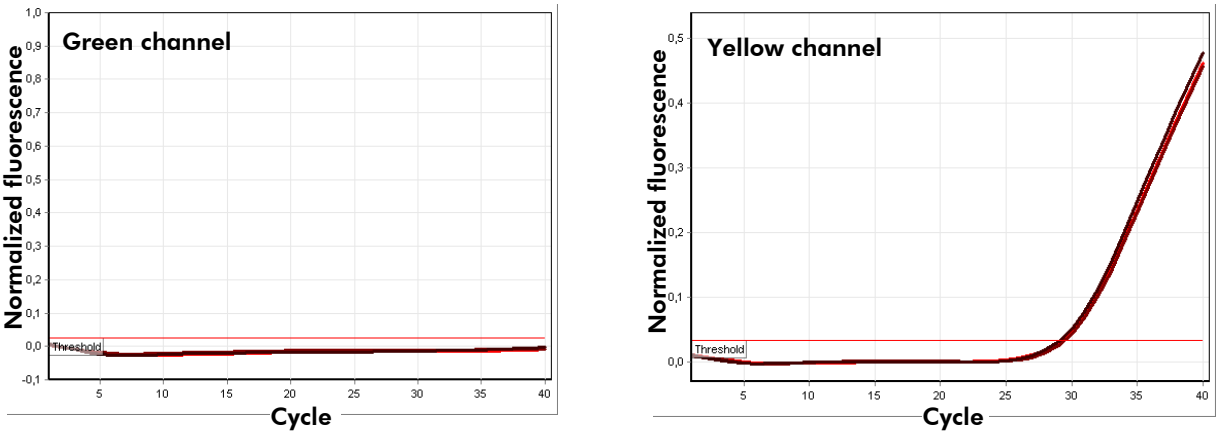

Figure 1. The sample is negative for the tested GMO or genetic construct. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was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

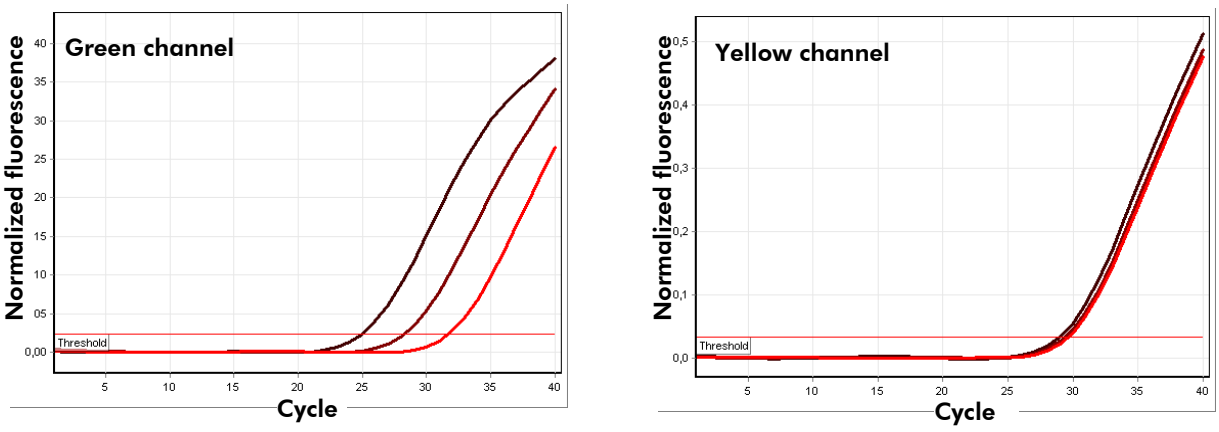

Figure 2. The sample is positive for the tested GMO or genetic construct. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of target DNA in the sample and a successful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

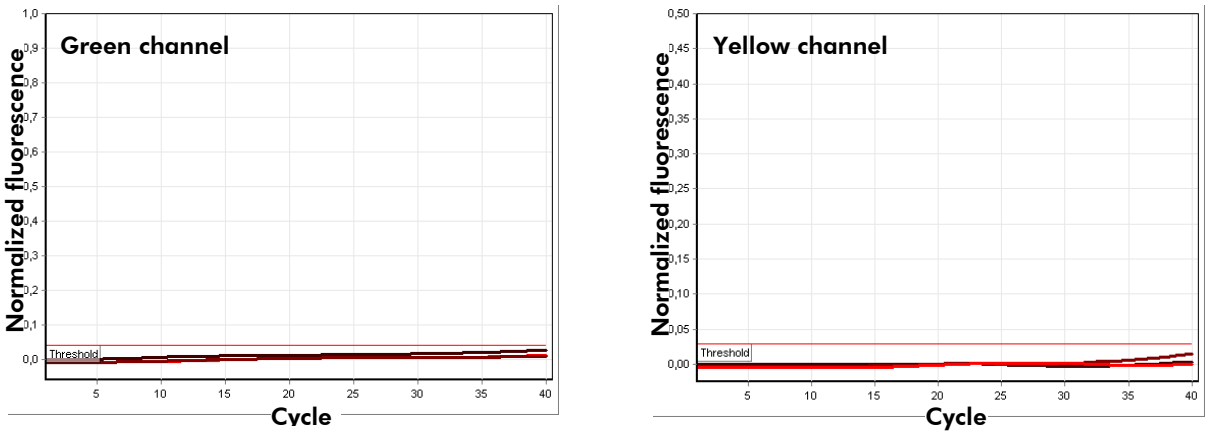

Figure 3. The PCR is inhibited. There was no amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 17. Summary of possible outcomes

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

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 quantification cycle (C_q) values. As a guideline, the uninhibited Internal Control should give a quantification cycle value ranging between 28 and 32. A quantification cycle value above 33 indicates inhibition.

In the event of PCR inhibition, dilute the extracted samples 1:10 with RNase-free water and repeat the test.

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

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
No signal with positive 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. See Table 11 or Table 12 on page 24, or Table 16 on page 28. For Applied Biosystems software, the channel for the internal control is specified during calibration with MAX NHS Ester dye. 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 Table 11 or Table 12 on page 24, or Table 16 on page 28. 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 23), Table 14 (page 26), or Table 15 (page 27). Repeat the PCR, if necessary.
d) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 6)	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 storage conditions and the expiration date (see the kit label) of the reagents and use a new kit, if necessary.

	Comments and suggestions
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 on page 24, or Table 16 on page 28. 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 provides a complete and efficient workflow for food safety testing.
c) The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 6)	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 storage conditions and 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 the 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 the work space and instruments are decontaminated at regular intervals.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

Ordering Information

Product	Contents	Cat. no.
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
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 GMO Screen 35S-pat Kit (24)*	For 24 reactions: PCR Assay Screen 35S-pat, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291023
mericon GMO Screen CTP2-CP4EPSPS Kit (24)*	For 24 reactions: PCR Assay Screen CTP2-CP4EPSPS, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291053
mericon GMO Screen bar Kit (24)*	For 24 reactions: PCR Assay Screen bar, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291063
mericon Mon 810 Corn Kit (24)*	For 24 reactions: PCR Assay MON 810 Corn, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291073

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
Related products		
***mericon* Quant GMO Detection Assays**		
mericon Quant RR Soy (48)	For 48 reactions: PCR Assay Quant RR Soy, PCR Assay Ref. System Quant RR Soy, Quant Control DNA Quant RR Soy, Standard DNA Quant RR Soy, 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 Quant MON 810, PCR Assay Ref. System Quant MON 810, Quant Control DNA Quant MON 810, Standard DNA Quant MON 810, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291524
***mericon* Pathogen Detection Assays**		
mericon Listeria spp Kit(24)*	For 24 reactions: PCR Assay Listeria spp, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	290123
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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
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
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

Product	Contents	Cat. no.
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
***mericon* Animal and Plant Identification Assays**		
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 Corn Kit (24)*	For 24 reactions: PCR Assay Corn, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293023
mericon Apricot Kernel Kit (24)*	For 24 reactions: PCR Assay Apricot Kernel, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293033
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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
mericon Cattle Kit (24)*	For 24 reactions: PCR Assay Cattle, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292023
mericon Chicken Kit (24)*	For 24 reactions: PCR Assay Chicken, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292033
mericon Turkey Kit (24)*	For 24 reactions: PCR Assay Turkey, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292043
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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

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