

April 2013

***mericon*[™] Plant and Animal Identification Handbook**

For identification of plant and animal matter in food or animal
feed samples using real-time PCR



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

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Kit Contents

mericon Plant and Animal Identification Assays		(24)	(96)
See Table 1 for specific kits			
Number of reactions		24	96
Yellow	<i>mericon Assay</i> *	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	210 µl
	Quick-Start Protocol	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 Plant and Animal Identification Assays

Product name	Catalog no.	
	(24)	(96)
<i>mericon</i> Soy Kit For specific detection of food components made from soy*	293013	293045
<i>mericon</i> Corn Kit For specific detection of food components made from corn*	293023	293025
<i>mericon</i> Apricot Kernels Kit For specific detection of food components made from apricot kernels*	293033	293035
<i>mericon</i> Pig Kit For specific detection of food components of pig origin*	292013	292015
<i>mericon</i> Cattle Kit For specific detection of food components of cattle origin*	292023	292025
<i>mericon</i> Chicken Kit For specific detection of food components of chicken origin*	292033	292035
<i>mericon</i> Turkey Kit For specific detection of food components of turkey origin*	292043	292045
<i>mericon</i> Ruminants Kit For specific detection of food components of ruminant origin*	292073	292075
<i>mericon</i> Sheep Kit For specific detection of food components of sheep origin*	292063	292065
<i>mericon</i> Goat Kit For specific detection of food components of goat origin*	292053	292055
<i>mericon</i> Horse Kit' For specific detection of food components of horse origin*	292143	292145

* For more detailed information, see "Assay-specific information", starting page 11.

Storage

mericon Plant and Animal Identification 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 $2-8^{\circ}\text{C}$ 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* Plant and Animal Identification Assays should be dispensed into aliquots to avoid more than 5 freeze–thaw cycles, and stored at $2-8^{\circ}\text{C}$ for short-term storage (1 month) or -20°C for long-term storage.

Product Use Limitations

mericon Plant and Animal Identification 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* Plant and Animal Identification 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* Plant and Animal Identification 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 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.

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 Plant and Animal Identification Assays are a ready-to-use system for the detection of DNA sequences from specific animal or plant material found 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 plant- or animal-specific target sequence and an internal control (IC), plus probes labeled with 2 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 plant or animal material 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 a unique and conserved DNA region of the tested plant or animal that is verified bioinformatically and experimentally. Cross-reactivity has been bioinformatically investigated and 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 the *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 suitable for PCR instruments requiring a low ROX Dye concentration such as the Applied Biosystems models 7500 and 7500Fast. Instructions for using the dye are provided in the protocol “Detection of GMO DNA by real-time PCR with ROX” on page 28.

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®), the 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 Soy Kit

The *mericon* Soy Kit is designed for the detection of soy (*Glycine max*) in food, animal feed, and pharmaceutical products.

Limit of detection

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

Specificity

The *mericon* Soy Kit exhibits high specificity for soy and Roundup Ready soy. No cross-reactivity was observed with other plant or animal species or genetically modified organisms (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
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/mericonPathogens.

mericon Corn Kit

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

Limit of detection

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

Specificity

The *mericon* Corn Kit exhibits high specificity for corn. No cross-reactivity was observed with other plant or animal species (Table 3), using 50 ng of the tested DNA.

Table 3. Results from cross-reactivity experiment*

Species	Result	Species	Result
Corn	+	Sheep	–
Soy	–	Goat	–
Canola	–	Horse	–
Rice	–	Hazelnut	–
Potato	–	Pecan nut	–
Barley	–	Brazil nut	–
Wheat	–	Almond	–
Rye	–	Mustard	–
Cattle	–	Lupine	–
Pig	–		

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

mericon Apricot Kernels Kit

The *mericon* Apricot Kernels Kit is designed for the detection of apricot kernels in food, animal feed, and pharmaceutical products.

Limit of detection

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

Specificity

The *mericon* Apricot Kernels Kit exhibits high specificity for apricot. No cross-reactivity was observed with other plant or animal species (Table 4), using 50 ng of the tested DNA.

Table 4. Results from cross-reactivity experiment*

Species	Result	Species	Result
Apricot South Africa	+	Apricot China (sweet)	+
Apricot Turkey	+	Apricot Iran	+
Apricot Greece	+	Apricot Syria	+
Almond	–	Potato	–
Peach	–	Barley	–
Plum	–	Wheat	–
Rice	–	Rye	–
Cherry	–	Cattle	–
Hazelnut	–	Pig	–
Peanut	–	Sheep	–
Walnut	–	Goat	–
Pecan nut	–	Horse	–
Corn	–	Celery	–
Canola	–	Mustard	–
Soy	–	Lupine	–

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

mericon Pig Kit

The *mericon* Pig Kit is designed for the detection of domesticated pig (*Sus scrofa domestica*) and wild boar (*Sus scrofa*) matter in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Pig Kit exhibits high specificity for domesticated pig and wild boar. No cross-reactivity was observed with other animal species (Table 5), using 50 ng of the tested DNA.

Table 5. Results from cross-reactivity experiment*

Species	Result	Species	Result
Domesticated pig	+	Chicken	–
Wild boar	+	Sheep	–
Turkey	–	Goat	–
Duck	–	Cattle	–
Goose	–	Horse	–
Deer	–	Ostrich	–
Kangaroo	–	Reindeer	–
Moose	–	Cod	–
Salmon	–		

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

mericon Cattle Kit

The *mericon* Cattle Kit is designed for the detection of cattle matter (excluding water buffalo) in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Cattle Kit exhibits high specificity for cattle. No cross-reactivity was observed with other plant or animal species (Table 6), using 50 ng of the tested DNA.

Table 6. Results from cross-reactivity experiment*

Species	Result	Species	Result
Cattle	+	Deer	–
Water buffalo	–	Cod	–
Turkey	–	Salmon	–
Chicken	–	Rabbit	–
Goose	–	Corn	–
Duck	–	Soy	–
Pig	–	Rice	–
Sheep	–	Wheat	–
Goat	–	Potato	–
Horse	–		

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

mericon Chicken Kit

The *mericon* Chicken Kit is designed for the detection of chicken matter in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Chicken Kit exhibits high specificity for chicken. No cross-reactivity was observed with other animal species (Table 7), using 50 ng of the tested DNA.

Table 7. Results from cross-reactivity experiment*

Genetic construct, GMO, or species	Result	Genetic construct, GMO, or species	Result
Chicken	+	Deer	–
Turkey	–	Cod	–
Goose	–	Water buffalo	–
Duck	–	Salmon	–
Ostrich	–	Rabbit	–
Cattle	–	Corn	–
Pig	–	Soy	–
Sheep	–	Rice	–
Goat	–	Wheat	–
Horse	–	Potato	–

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

mericon Turkey Kit

The *mericon* Turkey Kit is designed for the detection of turkey matter in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Turkey Kit exhibits high specificity for turkey. No cross-reactivity was observed with other animal species (Table 8), using 50 ng of the tested DNA.

Table 8. Results from cross-reactivity experiment*

Species	Result	Species	Result
Turkey	+	Deer	–
Chicken	–	Cod	–
Goose	–	Water buffalo	–
Duck	–	Salmon	–
Ostrich	–	Rabbit	–
Cattle	–	Corn	–
Pig	–	Soy	–
Sheep	–	Rice	–
Goat	–	Wheat	–
Horse	–	Potato	–

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

mericon Ruminants Kit

The *mericon* Ruminants Kit is designed for the detection of ruminant matter in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Ruminants Kit exhibits high specificity for ruminants. No cross-reactivity was observed with other animal species (Table 9), using 50 ng of the tested DNA.

Table 9. Results from cross-reactivity experiment*

Species	Result	Species	Result
Cattle	+	Water buffalo	+
Sheep	+	Goat	+
Deer	+	Salmon	–
Turkey	–	Rabbit	–
Chicken	–	Corn	–
Goose	–	Soy	–
Duck	–	Rice	–
Pig	–	Wheat	–
Horse	–	Potato	–
Cod	–		–

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

mericon Sheep Kit

The *mericon* Sheep Kit is designed for the detection of sheep matter in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Sheep Kit exhibits high specificity for sheep. No cross-reactivity was observed with other animal species (Table 10), using 50 ng of the tested DNA.

Table 10. Results from cross-reactivity experiment*

Species	Result	Species	Result
Sheep	+	Water buffalo	–
Cattle	–	Goat	–
Deer	–	Salmon	–
Turkey	–	Rabbit	–
Chicken	–	Corn	–
Goose	–	Soy	–
Duck	–	Rice	–
Pig	–	Wheat	–
Horse	–	Potato	–
Cod	–		–

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

mericon Goat Kit

The *mericon* Goat Kit is designed for the detection of goat matter in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Goat Kit exhibits high specificity for goat. No cross-reactivity was observed with other animal species (Table 11), using 50 ng of the tested DNA.

Table 11. Results from cross-reactivity experiment*

Species	Result	Species	Result
Goat	+	Water buffalo	–
Cattle	–	Sheep	–
Deer	–	Salmon	–
Turkey	–	Rabbit	–
Chicken	–	Corn	–
Goose	–	Soy	–
Duck	–	Rice	–
Pig	–	Wheat	–
Horse	–	Potato	–
Cod	–		–

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

mericon Horse Kit

The *mericon* Horse Kit is designed for the detection of horse matter in food and in animal feed.

Limit of detection

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

Specificity

The *mericon* Horse Kit exhibits high specificity for horse. No cross-reactivity was observed with other animal species (Table 12), using 50 ng of the tested DNA.

Table 12. Results from cross-reactivity experiment*

Species	Result	Species	Result
Horse	+	Water buffalo	–
Donkey	+	Goat	–
Cattle	–	Salmon	–
Deer	–	Rabbit	–
Turkey	–	Chicken	–
Goose	–	Soy	–
Duck	–	Rice	–
Pig	–	Wheat	–
Sheep	–	Potato	–
Cod	–	Corn	–

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

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.

- 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 13 on page 24 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 cooled Loading 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 4, page 14 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 plant or animal 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 13 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 13. 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, 7500 Fast 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 13, 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 DNA from Specific Plants or Animals by Real-Time PCR without ROX

Important points before starting

- Before beginning the procedure, read “Important Notes”, page 23.
- 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 μ l 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 μ l 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 μ l 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 14, page 26. 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 14. Setup of sample and control reactions

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted <i>mericon</i> Assay	10 μ l	10 μ l	10 μ l
Sample DNA	10 μ l	–	–
Dissolved Positive Control DNA	–	10 μ l	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	10 μ l
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 15 (page 27). For all other real-time cyclers, use the cycling protocol in Table 16 (page 27).**

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 17 on page 28.

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 32.**

Table 15 . 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 16. 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.13, page 24, for information on instrument-specific detection channel or filter set.

Protocol: Detection of DNA from Specific Plants or Animals 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 23.
- 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 17. Mix by pipetting up and down 5 times or vortexing and centrifuge briefly.

Note: The 50x ROX Dye solution provided is suitable for PCR instruments, requiring a low ROX Dye concentration. For PCR instruments requiring a high ROX Dye concentration please contact QIAGEN Technical Service.

Table 17. Components to add to the *mericon* Assay

Thermal cycler	Kit size	ROX dye	Multiplex PCR Master Mix
Applied Biosystems model 7500 and 7500 Fast	24	5.4 μ l per vial	130 μ l per vial
	96	43.3 μ l	1040 μ l
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 18 (page 29). Keep all samples and reaction tubes on ice during setup.

Table 18. Setup of sample and control reactions for PCR instruments requiring ROX Dye at a low concentration

Component	Sample	Positive PCR control	Negative PCR control
Reconstituted <i>mericon</i> 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 19 (page 31).**
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 13 on page 24.
- 4. Start the PCR run.**
- 5. Proceed to the protocol “Analyzing the Results” on page 32.**

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	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 13, page 24, for information on 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 20 (page 21).

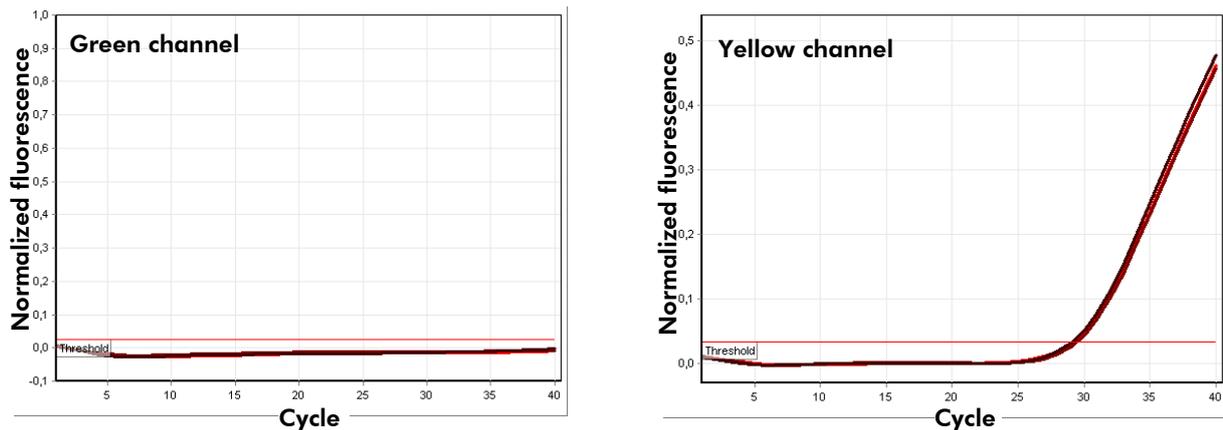


Figure 1. The sample is negative for the tested plant or animal. The 3 sample curves in the green channel (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

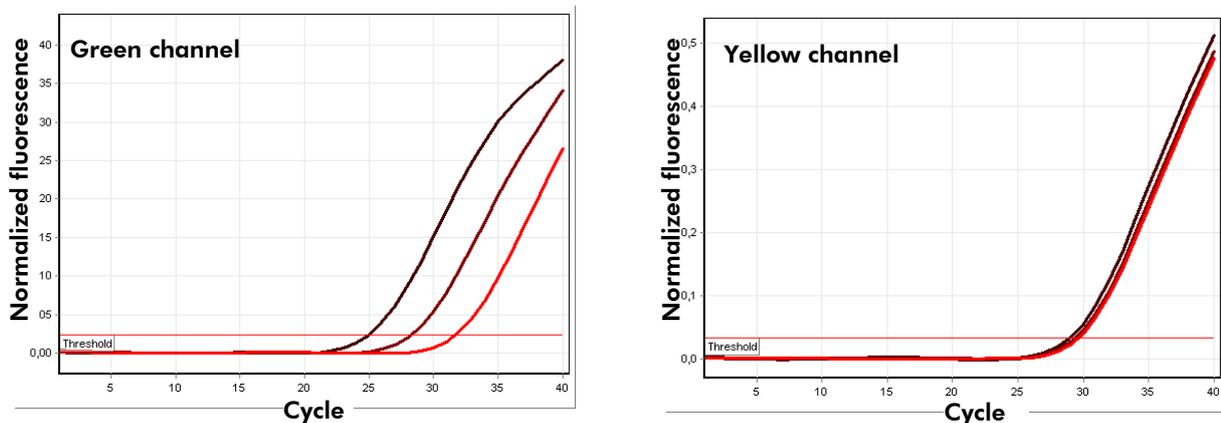


Figure 2. The sample is positive for the tested plant or animal. The 3 sample curves in the green channel (left) and the corresponding curves of the internal control in the yellow channel (right) are above a preset threshold indicating the presence of target DNA in the sample and a successful PCR.

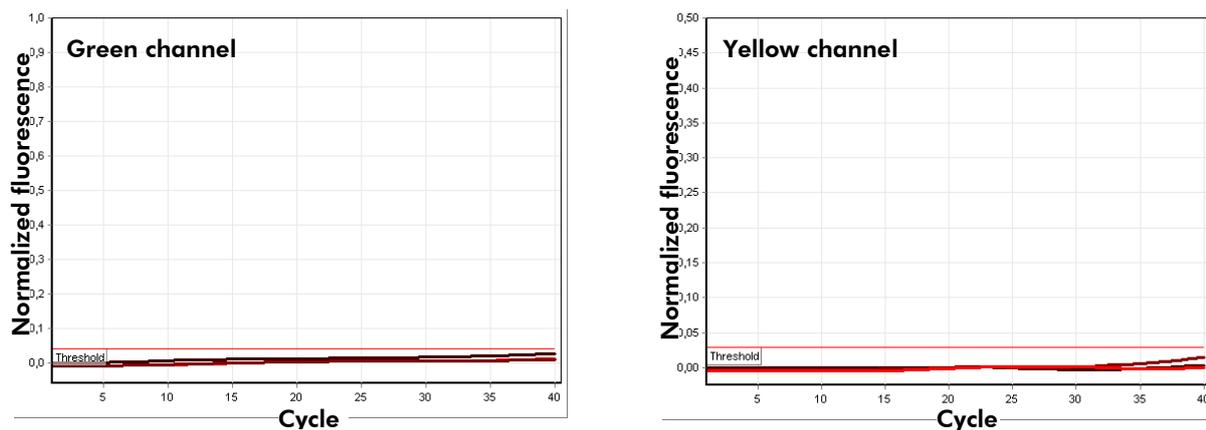


Figure 3. The PCR is inhibited. There is no amplification of the three samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 20 . Summary of possible outcomes

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

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 thresholds (C_T) values. As a guideline, the uninhibited internal control should give a cycle threshold value ranging between 28 and 32. A cycle threshold 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 15 or Table 16 on page 27, or Table 19 on page 31. 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 15 or Table 16 on page 27, or Table 19 on page 31. 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 14. (page 26) or Table 18 (page 29). 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 <i>mericon</i> 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 15 or Table 16 on page 27, or Table 19 on page 32. 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 <i>mericon</i> 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 <i>mericon</i> 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.
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> Apricot Kernels Kit (24)*	For 24 reactions: PCR Assay Apricot Kernels, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	293033
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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

* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon</i> Ruminant 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	292073
<i>mericon</i> Sheep 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	292063
<i>mericon</i> Goat 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	292053
<i>mericon</i> Horse 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	292143
Related products		
<i>mericon</i> GMO Detection Assays		
<i>mericon</i> 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
<i>mericon</i> 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

Product	Contents	Cat. no.
<i>mericon</i> 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
<i>mericon</i> GMO Screen 35S-pat (24)*	For 24 reactions: PCR Assay GMO Screen 35S-pat, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291023
<i>mericon</i> GMO Screen CTP2-CP4EPS (24)*	For 24 reactions: PCR Assay GMO Screen CTP2-CP4EPS, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291053
<i>mericon</i> GMO Screen bar (24)*	For 24 reactions: PCR Assay GMO Screen bar, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	291063
<i>mericon</i> Mon 810 Corn (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
<i>mericon</i> Quant GMO Detection Assays		
<i>mericon</i> Quant Roundup Ready 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

* Larger kit sizes available; please inquire

Product	Contents	Cat. no.
<i>mericon</i> 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
<i>mericon</i> Pathogen Detection Assays		
<i>mericon</i> Listeria spp (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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> 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

Product	Contents	Cat. no.
<i>mericon</i> 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
<i>mericon</i> 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
<i>mericon</i> Quant Legionella spp Kit (96)*	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
<i>mericon</i> Quant L. pneumophila Kit (96)*	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
<i>mericon</i> 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
<i>mericon</i> 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
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DNeasy <i>mericon</i> Food Kit (50)	50 QIAquick® Spin Columns, Proteinase K, buffers	69514
<i>mericon</i> DNA Bacteria Kit (100)	Fast Lysis Buffer	69525

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* Larger kit sizes available; please inquire.

Product	Contents	Cat. no.
<i>mericon</i> DNA Bacteria Plus Kit (50)	50 Pathogen Lysis Tubes L, Fast Lysis Buffer	69534
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