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mericon® MeatTracker Kit Handbook

For detection of animal DNA in food, animal feed or pharmaceutical samples using real-time PCR

Sample to Insight

QIAGEN

Contents

Kit Contents

mericon **MeatTracker Kit (96)**	**(96)**
Catalog no.	**290145**
Number of reactions	**96**
mericon Assay* (tube with yellow cap)	96 reactions
Positive Control DNA (tube with red cap)	20 reactions
QuantiTect® Nucleic Acid Dilution Buffer	1.5 ml
RNase-free water	1.9 ml
Multiplex PCR Master Mix† (tube with blue cap)	1040 µl
50x ROX Dye Solution	210 µl
Quick-Start Protocol	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).

Storage

The *mericon* Assays are shipped on dry ice. Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer and ROX Dye Solution should be stored immediately at –15°C to –30°C upon receipt, in a constant-temperature freezer.

All remaining non-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.

Once reconstituted, reagents 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 –15°C to –30°C for long-term storage.

Intended Use

The *mericon* MeatTracker Kit is intended for molecular biology applications in food, animal feed and pharmaceutical product testing. This product is not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH, or other applicable guidelines that have been developed for recombinant DNA experiments.

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.

	**CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.**

Quality Control

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

General Precautions for Real-Time PCR Assays

The *mericon* MeatTracker Kit involves DNA detection by PCR. Care must be taken to avoid contamination of the PCR reactions.

It is extremely important to include at least one negative control that lacks the template nucleic acid in every PCR setup to detect possible contamination.

General physical and chemical precautions

- 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.
- Use a separate set of pipets for the PCR master mix and the DNA samples. Use of pipet tips with hydrophobic filters is strongly recommended.
- Use gloves and protective laboratory wear. Do not touch any PCR equipment and supplies (e.g., rotors, loading blocks, tubes, pipets) without wearing gloves.
- In case of contamination, laboratory benches, apparatus and pipets can be decontaminated by cleaning them with a 1/10 dilution of a commercial bleach solution. Afterwards, the benches and pipets should be rinsed with distilled water.

Assay-Specific Information

mericon MeatTracker Kit

The *mericon* MeatTracker Kit is designed for detection of animal DNA (meat species) in food, animal feed and pharmaceutical samples purified with the DNeasy® *mericon* Food Kit or a comparable DNA purification method. The detection of meat species is essential for origin verification and traceability of raw materials.

Limit of detection

The *mericon* MeatTracker Kit can detect as few as 10 copies of target DNA in a reaction.

Specificity

The *mericon* MeatTracker Kit exhibits high specificity for animal species DNA. The kit has been tested against an extensive inclusivity panel of various vertebrate species and an

exclusivity panel of non-vertebrate species. No cross-reactivity was observed with species using 2500 copies of tested DNA (Table 1). Information about full inclusivity/exclusivity test panels is available at **www.qiagen.com/products/food-safety-testing/ingredient-authentication/**.

Table 1. Results from cross-reactivity experiments with the *mericon* MeatTracker Kit

Species	Result
Cattle	+
Chicken	+
Goat	+
Goose	+
Horse	+
Moose	+
Ostrich	+
Pig Wild boar	+ +
Rabbit	+
Sheep	+
Turkey	+
Water buffalo	+
Corn	–
Potato	–
Rice	–
Soy	–
Wheat	–

Introduction

The *mericon* MeatTracker Kit is a ready-to-use system for the detection of specific DNA fragments from animal species in food, animal feed and pharmaceutical products using real-time polymerase chain reaction (PCR). The *mericon* MeatTracker Kit can for further analysis be combined with one or more of the *mericon* Animal ID Assays indicated in Table 2.

Table 2. *mericorn* **Animal ID Assays**

Assay	Catalog number	
Number of reactions	**24**	**96**
mericon Cattle Kit	292023	292025
mericon Chicken Kit	292033	292035
mericon Goat Kit	292053	292055
mericon Ruminant Kit	292073	–
mericon Horse Kit	292143	292145
mericon Pig Kit	292013	292015
mericon Sheep Kit	292063	292065
mericon Turkey Kit	292043	292045

The Multiplex PCR Master Mix 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. The analytical procedure of this protocol allows the user to perform analysis in accordance with local official requirements.

Each *mericon* assay is an optimized mixture of PCR primer sets for species-specific target detection 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 (*524/557* nm). In addition, each kit includes internal control DNA and all reagents necessary to perform the analysis.

Principle and Procedure

Animal DNA detection by the polymerase chain reaction (PCR) is based on the amplification of a specific region of the relevant animal genome. 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 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 species genome that has been 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 as few as 10 target copies of each target 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, thereby preventing 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.

50x ROX Dye Solution

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 "Protocol: Detection of Animal DNA by Real-Time PCR with ROX" on page 19.

Primer/probe mix with internal control

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

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 (cat. no. 69514) or QIAamp® Fast DNA Tissue Kit (cat. no. 51404). The QIAamp procedure can be fully automated on the QIAcube®.
- Pipets (adjustable)
- Sterile pipet tips with filters
- Rotor-Gene Q or other real-time PCR instrument* with fluorescence detection for approximately 520 nm (FAM) and approximately 560 nm (MAX).
- PCR plastics for the thermal cycler to be used
  For the Rotor-Gene Q:
  - Strip Tubes and Caps, 0.1 ml, for use with 72-well rotor (250 or 2500) (cat. no. 981103 or 981106) or Rotor-Disc® 72 (24 or 240) (cat. no. 981301 or 981303) and Rotor-Disc Heat Sealing Film (60 or 600) (cat. no. 981601 or 981604)
  - Loading Block 72 x 0.1 ml Tubes (cat. no. 9018901), Rotor-Disc 72 Loading Block (cat. no. 9018910), or Loading Block 96 x 0.2 ml Tubes, cat. no. 9018905
- Tube rack
- Microcentrifuge*
- Vortex mixer

* Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Important Notes

General precautions

Please refer to "General Precautions for Real-Time PCR Assays", page 6. The user should always pay attention to the following:

- Use gloves as well as sterile pipet tips with filters.
- 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* Animal ID Assay contains an internal control to detect possible PCR inhibition from food, feed and pharmaceutical samples.

Negative PCR control

Negative controls (non-template 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 RNase-free water.

Postive PCR control

The Positive Control DNA contains an artificial DNA molecule containing the target of the *mericon* MeatTracker Assay and other *mericon* Animal ID Assays. It serves as a positive control for the meat identification experiment.

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 3 lists common thermal cyclers with their calibration requirements 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 3. 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	Not required	Yellow
Applied Biosystems models 7000, 7300, 7500, 7500 Fast, 7900HT, StepOne™, StepOnePlus™, ViiA®7, Quantstudio® 12K Flex	Required for new instruments†	VIC
Stratagene (Agilent) models Mx3005P®, Mx3000P®, Lightcycler 480, Smartcycler models, Bio-Rad instruments	Not required	Filter set 535/550 nm (HEX, JOE, VIC)

* For information on detection channel settings for instruments not listed in Table 3, 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 Animal DNA by Real-Time PCR without ROX

Important points before starting

- Before beginning the procedure, read "Important Notes", page 14.
- 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).
  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 at –15°C to –30°C for long-term storage.
- Reconstitute the 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 reconstituted 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 should be thawed completely, mixed by pipetting up and down pipetting 5 times or vortexing and centrifuged briefly.
- Controls are assayed in single determination, while non-template controls (NTC) are assayed in duplicate.

Procedure

1. Set up the sample and control reactions according to Table 4. If you are working with the RGQ template files, check the plate layout in advance in order to choose similar positions for controls and unknown samples. Keep all samples and reaction tubes on a cooled rack during setup.

Table 4. Setup of sample and control reactions

Component	Samples	Positive PCR control	Negative control
Reconstituted *mericon* Assay	10 µl	10 µl	10 µl
Dissolved Positive Control DNA	–	10 µl	–
Sample DNA	10 µl	–	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	10 µl
Total volume	**20 µl**	**20 µl**	**20 µl**

2. Place the desired number of Strip Tubes, the Rotor-Disc, or other PCR plate into the appropriate cooled Loading Block.
3. Close the Strip Tubes, ring or PCR plate and place them in the appropriate rotor of the Rotor-Gene Q, or in the reaction chamber of the thermal cycler, according to the instrument manual.

   Note: If you are using the Rotor-Gene Q with Strip Tubes, empty positions in the rotor should be filled with empty tubes. Make sure that the Locking Ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.
4. Close the PCR tubes or strips and place them in the reaction chamber of the thermal cycler, securing them according to the instrument manual.
5. 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.
6. Program the thermal cycler. If using the Rotor-Gene Q or Rotor-Gene 6000, use the cycling protocol in Table 5 (page 18). For all other real-time cyclers, use the cycling protocol in Table 6 (page 18).

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 3 on page 15.

7. For the Rotor-Gene Q or Rotor-Gene 6000 make sure that 'Perform Optimisation Before 1st Acquisition' in the Gain optimisation menu is activated.
8. Start the PCR run.
9. Proceed to "Analyzing the Results" on page 22.

Table 5. Cycling protocol for Rotor-Gene Q

	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	
Annealing*	15 s	60°C*	* Data collection at 60°C for channels green and yellow
Extension	10 s	72°C	
Number of cycles	45		
Gain optimization before first acquisition at 60°C for channels green and yellow			

Note: To set the gain optimization, select the green and yellow channels in the drop-down menu of the "Auto-Gain Optimisation Setup" and click "Add". In the dialog box that opens, confirm the standard settings. Click "Perform Optimisation Before 1st Acquisition". Then close the window. Make sure that the tube at position 1 is not empty, since the gain optimization will be performed on this tube.

Table 6. Cycling protocol for real-time cyclers other than Rotor-Gene Q

	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	
Annealing†	23 s‡	60°C†	† Data collection at 60°C for FAM and MAX (VIC§)
Extension	10 s	72°C	
Number of cycles	45		

‡ 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 3, page 15, for information on instrument-specific detection channel or filter set.

Protocol: Detection of Animal DNA by Real-Time PCR with ROX

For certain real-time cyclers, the use of a ROX passive reference dye during 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 14.
- 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 the Quantification Control and at least one negative control are included per PCR run.

Things to do before starting

- Prepare the *mericon* Assay (tube with yellow lid).
  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 at –15°C to –30°C for long-term storage.
- Add ROX dye to the Multiplex PCR Master Mix according to Table 7, page 20.

Table 7. Components to add to the *mericon* Assay

Thermal cycler	ROX dye	Multiplex PCR Master Mix
Applied Biosystems model 7500, 7500 Fast, ViiA7, QuantStudio 12K Flex	43.3 µl	1040 µl
ABI PRISM 7000, Applied Biosystems model 7300, 7900, StepOne, StepOne Plus	High ROX*	1040 µl
Rotor-Gene models, Stratagene Mx models, LightCycler 480, SmartCycler models, Bio-Rad instruments	–	1040 µl

* 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 Services.

Procedure

1. Set up the sample and control reactions according to Table 8. Keep all samples and reaction tubes on a cooled rack during setup.

Table 8. Setup of controls and sample reactions for PCR instruments requiring ROX

Component	Quantification control	Samples	Negative control
Reconstituted *mericon* Assay	10.4 µl	10.4 µl	10.4 µl
Quantification Control DNA	9.6 µl	–	–
Sample DNA	–	9.6 µl	–
QuantiTect Nucleic Acid Dilution Buffer or RNase-free water	–	–	9.6 µl
Total volume	**20 µl**	**20 µl**	**20 µl**

2. Place the desired number of PCR tubes or the PCR plate into the appropriate cooled Loading Block.
3. Close the PCR tubes or plate and place them in the reaction chamber of the thermal cycler, securing them according to the instrument manual.
4. Program the real-time cycler according to Table 9, page 21.
5. Start the PCR run.
6. Proceed to "Analyzing the Results", page 22.

Table 9. Cycling protocol

	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	
Annealing*	23 s[†]	60°C*	* Data collection at 60°C for FAM and MAX (VIC[‡])
Extension	10 s	72°C	
Number of cycles	45		

[†] For some instruments, the shortest annealing time possible is longer than 23 s (in the range of 32 s). In this case, use the shortest annealing time permitted by the instrument.

[‡] See Table 3, page 15, for information on instrument-specific detection channel or filter set.

Analyzing the Results

Qualitative animal species detection

Determining the presence or absence of animal species DNA 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 show examples of possible outcomes, which are summarized in Table 10, page 23.

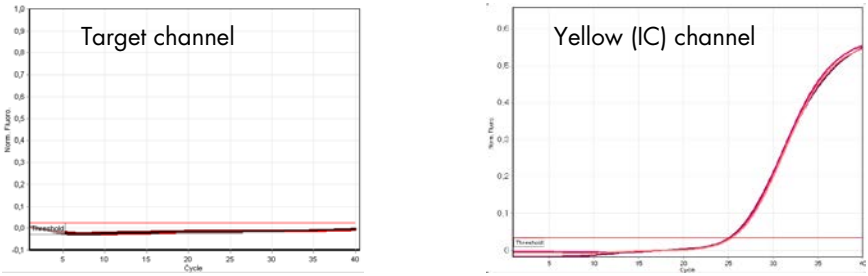

Figure 1. The sample is negative for tested animal species DNA. The 3 sample curves in the target channels (left) are at the baseline and below a preset threshold. The corresponding curves of the internal control in the yellow channel (right) are above the threshold, indicating that the PCR was successful.

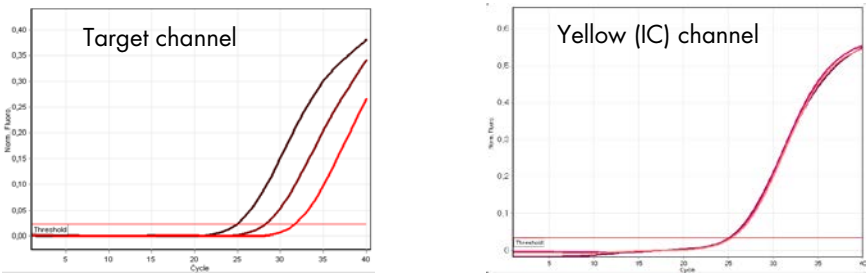

Figure 2. The sample is positive for tested animal species DNA. The 3 sample curves in the target channel (left) are above a preset threshold indicating the presence of target DNA. The corresponding curves of the internal control in the yellow channel (right) are above the threshold indicating that the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

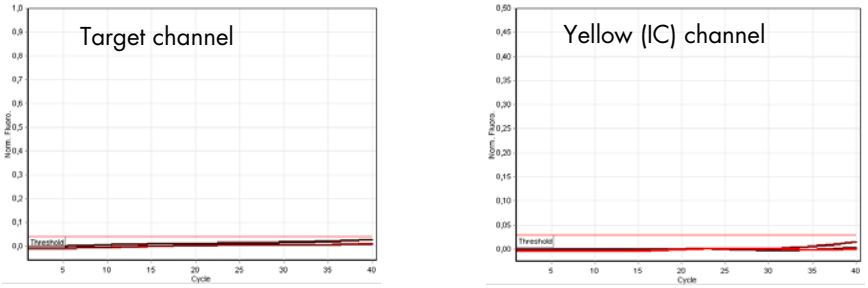

Figure 3. The PCR is inhibited. No amplification was observed for the 3 samples in the green channel (left) or the internal control in the yellow channel (right). All curves lie along the baseline and do not exceed a preset threshold.

Table 10. Summary of possible outcomes

Amplification of sample	Amplification of internal control	Result
+	+	Sample is positive
–	C_T ≤32*	Sample is negative
–	C_T >32* or No C_T	Internal control result is invalid, PCR inhibited; dilute sample and repeat test.

* C_T might be higher than 32 on real-time cyclers other than the Rotor-Gene Q. In these cases the C_T of control reactions (quantification control and negative control) serve as reference, and the C_T for the internal control in the samples reactions should not exceed +2.5 C_Ts.

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 threshold cycle (C_T) values. As a guideline, an uninhibited internal control should give a threshold cycle value below 32. A threshold cycle value above 32 indicates inhibition.

In the event of a PCR-inhibited internal control result, dilute the extracted samples 1:10 with RNase-free water and repeat the test.

If DNA template concentration is very high, a shift of the internal control to lower threshold cycle values might occur. This 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/or protocols in this handbook or sample and assay technologies (for contact information, 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 target and the yellow channel for the internal control. See the cycling protocols in Table 5, page 18, Table 6, page 18, or Table 9, page 21.
b) Incorrect programming of the real-time PCR instrument	Compare the temperature profile with the protocol. See the cycling protocols in Table 5, page 18, Table 6, page 18, or Table 9, page 21. Refer to the manufacturer's manual of the cycler to be used.
c) Incorrect configuration of the PCR	Ensure that reactions were set up according to Table 4, page 17, or Table 8, page 20. 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 5)	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.
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 5, page 18, Table 6, page 18, or Table 9, page 21. Repeat the PCR with corrected settings, if necessary.

b)	The PCR was inhibited	Use the recommended DNA isolation method and closely follow the manufacturer's instructions. QIAGEN offers dedicated sample preparation kits developed to complement *mericon* PCR Assays and provide a complete and efficient workflow for food safety testing. If there is still inhibition, dilute the DNA 1:10.
c)	The storage conditions for one or more kit components did not comply with the instructions given in "Storage" (page 5)	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 your positive controls last. Make sure that work space and instruments are decontaminated at regular intervals.
b)	Contamination occurred during extraction	Repeat the extraction and PCR of the sample to be tested using new reagents. Make sure that work space and instruments are decontaminated at regular intervals.

Ordering Information

Product	Contents	Cat. no.
mericon MeatTracker Kit (96)	For 96 reactions: PCR Assay Meat, Control DNA, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution (for use with *mericon* Animal ID Kits)	290145
Related products		
mericon **Animal ID Kits**		
mericon Pig Kit (96)*	For 96 reactions: PCR Assay Pig, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292015
mericon Cattle Kit (96)*	For 96 reactions: PCR Assay Cattle, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292025
mericon Horse Kit (96)*	For 96 reactions: PCR Assay Horse, Internal Control, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water	292145

* Other kit sizes available; please inquire.

Product	Contents	Cat. no.
mericon Sheep Kit (96)*	For 96 reactions: PCR Assay Sheep, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292065
mericon Goat Kit (96)*	For 96 reactions: PCR Assay Goat, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292055
mericon Chicken Kit (96)*	For 96 reactions: PCR Assay Chicken, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292035
mericon Turkey Kit (96)*	For 96 reactions: PCR Assay Turkey, Positive Control, Multiplex PCR Master Mix, QuantiTect Nucleic Acid Dilution Buffer, RNase-Free Water, 50x ROX Dye Solution	292045
***mericon* sample preparation kits**		
DNeasy *mericon* Food Kit (50)	50 QIAquick® Spin columns, Proteinase K, buffers	69514
QIAamp Fast DNA Tissue Kit (50)	50 QIAamp Spin Columns, QIAGEN Proteinase K, RNase A, Tissue Disruption Tubes, Buffers	51404

* Other kit sizes available; please inquire.

Product	Contents	Cat. no.
Instruments		
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	9001640
Rotor-Gene Q 2plex	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories, 1-year warranty on parts and labor	9001620
QIAcube	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits: includes 1-year warranty on parts and labor	9001293

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