Quick-Start Protocol November 2016

mericon® MeatTracker Kit

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.

Further information

- mericon *MeatTracker Kit Handbook*: **www.qiagen.com/handbooks**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: toll-free 00800-22-44-6000 or **www.qiagen.com/contact**

Notes before starting

- 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.
- The following procedure is for detection of animal DNA by real-time PCR without ROX 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. ROX dye is not necessary for the Rotor-Gene® Q. Instructions for using the dye are provided in the mericon *MeatTracker Kit Handbook*.

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.
- 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.
- 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.

Protocol

1. Set up the sample and control reactions according to Table 1. 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 1 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.
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 2. For all other real-time cyclers, use the cycling protocol in Table 3.
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. Analyze the results according to Table 4.

Table 2 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			

Table 3 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		

Table 4 Summary of possible outcomes

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

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