mericon® Ingredient Authentication Kits on the Rotor-Gene® Q

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. Reconstituted reagents of *mericon* Ingredient Authentication 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.

Further information

- mericon *Plant and Animal Identification Handbook*: ww.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

- The protocol below is for use with the Rotor-Gene Q. For other cyclers, please see the mericon *Plant and Animal Identification Handbook*.
- Use gloves as well as sterile pipet tips with filters.
- Ensure that at least one positive and negative control is included per PCR run.
- PCR tubes should be kept on ice until they are placed in the thermal cycler.
- Prepare the *mericon* Assay (tube(s) with yellow lid) and dried Positive Control DNA (tube with red lid). See the mericon *Plant and Animal Identification Handbook* for more information.
- Before each use, all reagents must be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing), and centrifuged briefly.

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1. Set up the sample and control reactions according to Table 1. Keep all samples and reaction tubes on ice during setup. Place the desired number of PCR tubes or strips into the cooled Loading Block for the Rotor-Gene Q.

Table 1. 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 Rotor-Gene Q. Ensure that the locking ring is placed on top of the rotor to prevent accidental opening of the tubes during the run.
3. Program the Rotor-Gene Q according to Table 2.

Table 2. Cycling protocol

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

4. Start the PCR run.
5. Analyze the results; see next page.

Interpretation of results

Determining the presence or absence of the target 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.

Table 3. 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.