

Quick-Start Protocol

mericon[®] Food Testing Assays for Cattle, Chicken, Sheep, and Pig on QIAcuity[®]

The *mericon* Assays (cat. nos. 1073122, 1067074, 1067064, and 1064506) and QIAcuity Probe PCR Mix (cat. no. 250101) are shipped on dry ice. The QIAcuity Probe PCR Mix (cat. no. 250101) should be stored immediately at -30 to -15°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, the 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 five freeze–thaw cycles, and stored at 2 – 8°C for short-term storage (1 month) or -30 to -15°C for long-term storage.

Further information

- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- Use gloves as well as sterile pipette 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.
- The following procedure is for detection of animal DNA by digital PCR (dPCR) on the QIAcuity dPCR platform.

- Users have the option to enzymatically fragment their DNA samples prior to dPCR. The Cattle, Pig, Sheep, and Chicken Assays and IC Assay are all compatible with EcoRI digestion. When added directly to the QIAcuity reaction mix, EcoRI-HF® from NEB® at a final concentration of 0.25 U/μL and Anza™ 11 EcoRI (Thermo Fisher Scientific) at a final concentration of 0.025 U/μL will fully digest genomic DNA within 10 min at room temperature (15–25°C).
- Controls are assayed in single determination, while non-template controls (NTC) are assayed in duplicate.

Things to do before starting

- Reconstitute the lyophilized *mericon* Assay (tube with **YELLOW** lid).
 - First thaw the 4x QIAcuity Probe PCR Mix at room temperature. Mix by pipetting repeatedly up and down or by pulse vortexing.
 - Briefly centrifuge the *mericon* Assay tube to settle the dry oligonucleotides.
 - To reconstitute the lyophilized *mericon* Assay, add 315 μL of the 4x QIAcuity Probe PCR Mix to the *mericon* Assay tube. Mix by pipetting repeatedly up and down or by pulse vortexing.
- Reconstitute the Positive Control DNA (tube with **RED** lid).
 - First centrifuge the tube containing the Positive Control DNA to settle the lyophilized template.
 - Add 200 μL of water to the vial and mix by pipetting repeatedly up and down or by pulse vortexing. Centrifuge briefly to settle the liquids.

Procedure

1. Thaw the reconstituted *mericon* Assay, reconstituted Positive Control DNA, and sample DNA at room temperature. Mix the thawed components individually by pipetting repeatedly up and down or by pulse vortexing. Centrifuge briefly to settle the liquids.
2. Prepare reactions in a standard 96-well PCR plate according to Table 1.

3. First dispense the appropriate volume of reconstituted *mericon* Assay into the desired number of wells of a standard PCR plate. Each well corresponds to one dPCR reaction.
4. To wells containing reconstituted *mericon* Assay, add sample DNA, reconstituted Positive Control DNA, and/or RNase-free water. Thoroughly mix the contents of each well by gently pipetting up and down.

Note: If restriction enzyme for DNA digestion is included in the reaction, reduce the volume of template and/or RNase-free water accordingly and leave the plate for 10 min at room temperature.

5. Transfer the entire contents of each well from the standard PCR plate into the wells of a fresh nanoplate.
6. Seal the nanoplate properly using the QIAcuity Nanoplate Seal provided in the QIAcuity Nanoplate Kits.

Note: For exact sealing procedure, please see the *QIAcuity System User Manual*.

Table 1. Setup of sample and control reactions

Component	8.5k Nanoplate			26k Nanoplate		
	Sample	Positive PCR Control	No Template Control	Sample	Positive PCR Control	No Template Control
Reconstituted <i>mericon</i> Assay	3 µL	3 µL	3 µL	10 µL	10 µL	10 µL
Reconstituted Positive Control DNA	–	9 µL	–	–	30 µL	–
Sample DNA	up to 9 µL	–	–	up to 30 µL	–	–
EcoRI Restriction Enzyme (optional)	variable	variable	variable	variable	variable	variable
RNase-free water	variable	–	9 µL	variable	–	30 µL
Total volume	12 µL	12 µL	12 µL	40 µL	40 µL	40 µL

Thermal cycling conditions

1. Program the cycler of the QIAcuity instrument using the cycling conditions provided in Table 2 and imaging settings according to Table 3.
2. Place the nanoplate into the QIAcuity instrument and start the dPCR program.

Table 2. Cycling conditions

Step	Time	Temperature (°C)
PCR initial heat activation	2 min	95
3-step cycling (40 cycles)		
Denaturation	15 s	95
Annealing	15 s	54
Extension	10 s	72

Table 3. Imaging Settings

Channel	Exposure Duration	Gain
Green	500 ms	6
Yellow	500 ms	6

Document Revision History

Date	Changes
09/2023	Initial release
11/23	Corrected QR code to link to



Scan QR code for the *QIAcuity User Manual*.

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