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QIAcuity® Mycoplasma Quant Kit Handbook

For the detection of mycoplasma using the QIAcuity dPCR System with Internal Control as RT-PCR control and preparation of Mycoplasma Standard CFU Kits

Sample to Insight

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Kit Contents

QIAcuity® Mycoplasma Quant Kit Catalog number Number of reactions		250261 96*
Component	**Quantity**	**Cap color**
4× OneStep Advanced Probe Master Mix	1 × 1 mL	Red
100× OneStep Advanced RT Mix	1 × 0.045 mL	Purple
OneStep Enhancer GC	1 × 1 mL	Yellow
20× QIAcuity Mycoplasma Assay	1 × 0.2 mL	Blue
QIAcuity Mycoplasma Internal Control, lyophilized	2 vials	Orange
QIAcuity Mycoplasma Positive Control, lyophilized	1 vial	Green
RNase-free Water	2 × 1.9 mL	Neutral

* The number of reactions is calculated based on 40 µL QIAcuity dPCR reactions.

Mycoplasma Standard CFU Kit **Catalog number** **Volume of Standard**		**250262–250271*** **3 mL**†
Component	**Quantity (vials)**	**Cap color**
QIAcuity Mycoplasma Std. CFU, lyophilized	3	Black
QIAcuity Mycoplasma Negative Control, lyophilized	1	Purple

* All available Mycoplasma 10 CFU Standard Kits are listed in the next table.

† The volume of standard is calculated based on recommended reconstitution with 1 mL matrix leading to a final concentration of 10 CFU/mL.

Mycoplasma Standard CFU Kits	**Catalog number**
Mycoplasma arginini Standard CFU Kit	250262
Mycoplasma orale Standard CFU Kit	250263
Mycoplasma gallisepticum Standard CFU Kit	250264
Mycoplasma pneumoniae Standard CFU Kit	250265
Mycoplasma synoviae Standard CFU Kit	250266
Mycoplasma fermentans Standard CFU Kit	250267
Mycoplasma hyorhinis Standard CFU Kit	250268
Acholeplasma laidlawii Standard CFU Kit	250269
Spiroplasma citri Standard CFU Kit	250270
Mycoplasma salivarium Standard CFU Kit	250271

Shipping and Storage

The QIAcuity Mycoplasma Quant Kit is shipped on dry ice.

The QIAcuity Mycoplasma Quant Kit should be stored protected from light at –30°C to –15°C upon receipt. Under these conditions, the components are stable until expiry date printed on their labels without showing any reduction in performance and quality, unless otherwise indicated on the labels.

The QIAcuity Mycoplasma Internal Control (before reconstitution), QIAcuity Mycoplasma Positive Control (before reconstitution), and RNase-free Water can be stored at 2–8°C (as indicated on the label) or at –30°C to –15°C.

After reconstitution, QIAcuity Mycoplasma Internal Control and QIAcuity Mycoplasma Positive Control are stable at –30°C to –15°C or 2–8°C for up to 8 months or until the expiry date printed on their labels, whichever occurs first. More than 5 freeze–thaw cycles should be avoided. If QIAcuity Mycoplasma Internal Control and QIAcuity Mycoplasma Positive Control were aliquoted, using DNA low-binding tubes is strongly recommended to minimize loss of material due to binding to plastic.

The Mycoplasma Standard CFU Kits are shipped on gel packs.

Upon receipt, the Mycoplasma Standard CFU Kits should be stored at 2–8°C. Under these conditions, the components are stable until expiry date printed on their labels without showing any reduction in performance and quality, unless otherwise indicated on the labels.

Intended Use

The QIAcuity Mycoplasma Quant Kit is intended for research use only and not for use in diagnostic procedures.

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 guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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 components.

Quality Control

In accordance with ISO-certified Quality Management System of QIAGEN, each lot of the QIAcuity Mycoplasma Quant Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAcuity Mycoplasma Quant Kit reliably detects and quantifies the presence of mycoplasma rRNA and DNA in a variety of sample types.

For a pharmacopoeia-compliant and validated workflow, refer to the *QIAcuity Mycoplasma Quant Kit Handbook* "for the detection of mycoplasma using the QIAcuity dPCR System with Spike-In Internal Control during sample preparation".

Principle and procedure

The QIAcuity Mycoplasma Quant Kit, together with the QIAcuity instrument, forms a system for detection and quantification of mycoplasma contamination designed for use with the QIAcuity instrument.

The workflow consists of 2 steps in which the nucleic acids are extracted from the sample and subsequently analyzed via reverse transcription digital PCR (RT-dPCR). Using the QIAcuity OneStep Advanced Probe chemistry, the required reverse transcription and PCR are done within a single hands-on step. Although we recommend detecting mycoplasma using the RT-dPCR protocol as described below, the assay also detects DNA and can be used in a dPCR without the RT step. We recommend conducting sample isolation for RNA and DNA.

Sample preparation

Elimination of PCR inhibitors and other impurities is required to have a reliable detection of any mycoplasma contamination within the sample. The eluate can be used directly for mycoplasma detection using the QIAcuity Mycoplasma Quant Kit with the corresponding QIAcuity OneStep Advanced Probe Chemistry. We recommend to use a sample isolation method for RNA and DNA.

An alternative workflow using an internal control added during sample preparation is described in a separate *QIAcuity Mycoplasma Quant Kit Handbook*.

QIAcuity OneStep Advanced Probe Chemistry

The HotStart Reverse Transcription (RT) Enzyme, in combination with the HotStart QuantiNova® DNA Polymerase and proprietary chemical components, enables an optimal reverse transcription and subsequent PCR for detection of any mycoplasma contamination using the QIAcuity Mycoplasma Assay without the need for a separate RT-reaction setup.

QIAcuity Mycoplasma Assay

The QIAcuity Mycoplasma Assay is provided as FAM™-labeled 20× primer–probe mix. Also included is a HEX-labeled primer–probe mix specific to the optional QIAcuity Mycoplasma Internal Control. This duplex reaction is proven for cross-talk and enables an overall control for RNA extraction, reverse transcription, amplification, and detection without setting up any extra reaction.

Description of protocols

This handbook includes a protocol for the detection of mycoplasma contamination with the use of the QIAcuity Mycoplasma Internal Control as RT-dPCR control only. A recommendation for the extraction method is given as the sample preparation method is crucial for the successful detection. A second protocol describes the use of Mycoplasma Standard CFU Kits.

The protocols described in this handbook are provided for assay use and in house testing purposes and are not intended to describe a validated pharmacopeia compliant workflow.

Detection of Mycoplasma using QIAcuity Mycoplasma Quant Kit with the Internal Control as RT-PCR control

Detailed description of reaction setup for the mycoplasma RNA quantification using the QIAcuity dPCR System with the use of the QIAcuity Mycoplasma Internal Control as RT-dPCR control only.

Preparation of Mycoplasma Standard CFU Kits

Detailed description of use of Mycoplasma Standard CFU Kits for in-house validation or as positive control for sensitivity of mycoplasma testing without introducing vital mycoplasma.

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.

- Mycoplasma Standard CFU kits (cat. nos. 250262–250271), optional
- QIAcuity Nanoplates (cat. no. 250001)
- Microcentrifuge tubes or PCR plates or strip tubes with appropriate sealing foil or cap.
- Single-channel or multichannel pipettor (manual or automatic) with nuclease-free, aerosol-barrier pipette tips
- Vortexer
- Centrifuge for tubes and plates
- QIAcuity One (5plex), Four, or Eight (cat. nos. 911021, 911042, or 911052)

Important Notes

Mycoplasma testing

Mycoplasma is a common contaminant of mammalian cell cultures. This contamination is not detectable by microscopy due to the very small size but can significantly affect the vitality and gene expression of mammalian cells, which affects any cell-derived products such as adeno-associated viruses (AAVs), proteins, or vaccines.

Starting material

The QIAcuity Mycoplasma Quant Kit is optimized for detecting the presence of mycoplasma nucleic acids. Test material can contain various matrices such as PBS, cell culture media like Dulbecco's modified Eagles medium (DMEM) with fetal calf serum (FCS) in the presence or absence of cell background, final ATMPs (advanced therapeutic medicinal products) like gene therapy tools or vaccines in different production steps. In combination with a suitable extraction method such as the EZ1&2® Virus Mini Kit v2.0 (48) (cat. no. 955134) or the Venor® Mycoplasma Extraction Kit (formerly known as Venor®GeM SP Kit – Beads, Minerva Biolabs GmbH, cat. nos. 56-3010 and 56-3100), the QIAcuity Mycoplasma Quant Kit is resistant against potential inhibitors such as coating agents, secreted intracellular, and extracellular material carried over by tested cell suspensions like HEK293 cells or CHO cells.

Protocol: Detection of Mycoplasma Using QIAcuity Mycoplasma Quant Kit with the Internal Control as RT-PCR Control

This protocol describes how to set up a QIAcuity RT-PCR using the QIAcuity Mycoplasma Quant Kit for mycoplasma detection in test samples processed by an appropriate nucleic acid purification method without the prior addition of the QIAcuity Mycoplasma Internal Control during the sample lysis.

Important points before starting

- See "Important Notes" on the previous page.
- The protocol for the QIAcuity Mycoplasma Quant Kit with the Internal Control as RT-PCR control uses a 10-fold lower concentration for QIAcuity Mycoplasma Internal Control than in the previous protocol to enable a correct pipetting volume.
- The 4× QIAcuity OneStep Advanced Probe Master Mix contains HotStart QuantiNova DNA polymerase, which is inactive at ambient temperature (18–25°C). The PCR protocol must start with a mandatory initial incubation step of 2 min at 95°C to activate the enzyme.
- The 100× QIAcuity OneStep Advanced RT Mix contains a HotStart Reverse Transcription (RT) Enzyme. This enzyme is inactive at ambient temperature, allowing users to assemble up to 4 or 8 plates and run them in parallel on QIAcuity Four or QIAcuity Eight instrument, respectively. Nevertheless, during reaction setup, 100× QIAcuity OneStep Advanced Reverse Transcription Mix should be stored on ice (2–8°C) to ensure constant performance.
- A fluorescent dye is provided as a component of the QIAcuity Probe PCR Master Mix for reliable detection of proper filling in the dPCR plates.

- Pipetting accuracy and precision affect the consistency of quantification results. Make sure that no air bubbles are introduced into the wells of the dPCR nanoplates during pipetting.

Things to do before starting

- Have an appropriate nucleic acid purification method ready to use. For manual extraction, QIAGEN recommends the EZ1&2 Virus Mini Kit v2.0 (48) for automated nucleic acid purification or the Venor Mycoplasma Extraction Kit (Minerva Biolabs GmbH) that allows for manual or automated nucleic acid purification. Alternative solutions can be used after performance assessment. Be sure to choose a solution that isolates total nucleic acid from the sample.
- Prepare all components of the nucleic acid purification method according to the respective protocol including preheating of potentially required heat blocks.
- Perform nucleic acid purification as described in the respective protocol and store the eluate for reaction setup in step 2 of this protocol on ice.
- If not already used as overall control of the procedure (nucleic acid purification, reverse transcription, digital PCR) as described in *QIAcuity Mycoplasma Quant Kit Handbook* "For the detection of mycoplasma using the QIAcuity dPCR System with the Spike-In Internal Control during Sample Preparation", reconstitute lyophilized QIAcuity Mycoplasma Internal Control by briefly centrifuging the tubes to remove any material from the lid. Dissolve lyophilized QIAcuity Mycoplasma Internal Control (720,000 copies) by adding 1200 µL RNase-free Water to reach a final concentration of 600 copies/µL. Incubate for 5 min at ambient temperature, then vortex and spin the reconstituted reagents.

  Note: QIAcuity Mycoplasma Internal Control can be stored at –15°C to –30°C or at 2–8°C for up to 8 months. Avoid more than 5 freeze–thaw cycles.
- Reconstitute lyophilized QIAcuity Mycoplasma Positive Control: Briefly centrifuge the tubes to remove any material from the lid. Dissolve lyophilized QIAcuity Mycoplasma Positive

Control (80,000 copies) by adding 400 µL RNase-free Water to reach a final concentration of 200 copies/µL. Incubate for 5 min at ambient temperature, then vortex and spin the reconstituted reagents.

Note: QIAcuity Mycoplasma Positive Control can be stored at –15°C to –30°C or at 2–8°C for up to 8 months. Avoid more than 5 freeze–thaw cycles.

- Thaw all kit components except the 100× QIAcuity OneStep Advanced Reverse Transcription Mix and mix all components (also components stored at ambient temperature and on ice) right before use.
- Place the 100× QIAcuity OneStep Advanced Reverse Transcription Mix on ice.

Procedure

1. Prepare the RT-dPCR reaction mix using QIAcuity Mycoplasma Quant Kit according to Table 1 in a standard PCR plate.

   Note: The use of QIAcuity Mycoplasma Internal Control is highly recommended to check the presence of inhibitors in the sample.

2. Add the eluate obtained from the sample preparation. We recommend to add the maximum volume of 22.6 µL to the reaction, but this can be adjusted.

   Note: You can add 10 µL of the reconstituted QIAcuity Mycoplasma Positive Control instead of the eluate to one or more wells to check the reaction performance. Adjust the volume by adding water accordingly.

Table 1. PCR setup

Component	Volume in Nanoplate 26k (8-well and 24-well)	Final concentration in reaction
4× OneStep Adv. Probe Master Mix	10 µL	1×
100× OneStep Adv. RT Mix	0.4 µL	1×
OneStep Enhancer GC	5 µL	–
20× QIAcuity Mycoplasma Assay	2 µL	1×
QIAcuity Mycoplasma Internal Control (if not used as Spike-in control)	1 µL*	15 copies/µL†
Sample‡ or QIAcuity Mycoplasma Positive Control	up to 22.6 µL sample or 10 µL QIAcuity Mycoplasma Positive Control	50 copies/µL§
RNase-free Water	Fill up to 40 µL	–
Total reaction volume	**40 µL**	**–**

* Add QIAcuity Mycoplasma Internal Control during RT-PCR setup only if it was not added already during nucleic acid purification. Otherwise, misleading results will be obtained.

† Exemplary volume of the QIAcuity Mycoplasma Internal Control. If 1 µL QIAcuity Mycoplasma Internal Control is added, a final concentration of 15 copies/µL is expected. The final concentration can be calculated using the formula

$$C_{\mathrm{IC}} = \frac{C_{\mathrm{start}} \times V_{\mathrm{IC}}}{V_{\mathrm{total}}}$$

where C_{IC} is the IC concentration in dPCR, C_{start} is the starting concentration, V_{IC} is the IC volume added to the reaction, and V_{total} is the total reaction volume.

‡ Sample extract should be generated using an appropriate total nucleic acid purification technique. For recommendations, see "Protocol: Detection of Mycoplasma Using the QIAcuity Mycoplasma Quant Kit with the Use of the Spike-In Internal Control During Sample Preparation".

§ Exemplary volume of the QIAcuity Mycoplasma Positive Control. If 10 µL QIAcuity Mycoplasma Positive Control are added, a final concentration of 50 copies/µL is expected. The final concentration can be calculated using the formula

$$C_{\mathrm{PC}} = \frac{C_{\mathrm{start}} \times V_{\mathrm{PC}}}{V_{\mathrm{total}}}$$

where C_{PC} is the PC concentration in dPCR, C_{start} is the starting concentration, V_{PC} is the PC volume added to the reaction, and V_{total} is the total reaction volume.

3. Seal the plate and mix thoroughly by vortexing the reaction mix 5 times, 1 s each. Spin down the plate by briefly centrifuging.
4. Transfer the content of each well to a 26k nanoplate avoiding bubbles. Seal the nanoplate and load it into the QIAcuity instrument. Start the run.

Thermal cycling and imaging conditions

1. In the QIAcuity Software Suite or on the QIAcuity instrument, under the dPCR parameters, set the cycling conditions according to Table 2.

Table 2. Cycling conditions

Step	Time	Temperature (°C)
Reverse Transcription reaction		
Reverse transcription	40 min	50
RT Enzyme inactivation	2 min	95
2-step cycling (40 cycles)		
Denaturation	15 s	95
Combined annealing and elongation	1 min	59

2. Under the dPCR parameters in the QIAcuity Software Suite or on the QIAcuity instrument, activate all needed channels in Imaging. Start with the default imaging settings according to Table 3.

Table 3. Imaging settings*

Channel	Exposure (ms)	Gain
Green (FAM), Mycoplasma	500	6
Yellow (HEX), Internal Control	500	6

* Imaging settings might need to be adjusted according to the assay. Always start with the recommended settings.

3. Load the Nanoplate into the QIAcuity instrument and start the dPCR program.

Data analysis

1. To set up a plate layout according to the experimental design, open the QIAcuity Software Suite and define the reaction mixes, samples, and controls. Plate layout can be defined before or after the Nanoplate run.

   Note: Refer to the *QIAcuity User Manual* for details on setting up the plate layout.

2. After the run is completed, the raw data are automatically sent to the QIAcuity Software Suite.

3. For data analysis, open the QIAcuity Software Suite and select the individual Nanoplate for the analysis in **Plate Overview** of the Software Suite.

   Note: See the *QIAcuity User Manual Extension: Application Guide* and *QIAcuity User Manual* for details on how to analyze absolute quantification data. Also, see the Validation Report *Evaluation of the mycoplasma detection capability of the QIAcuity Mycoplasma Quant Kit* for more details and result interpretation.

Additional technical and performance information is available in separate technical documentation.

Protocol: Preparation of Mycoplasma Standard CFU Kits

This protocol describes the preparation of Mycoplasma Standard CFU Kits and the use of the QIAcuity Mycoplasma Quant Kit in conjunction with the QIAcuity Mycoplasma Internal Control as spike-in during nucleic acid extraction to be used as overall control described in *QIAcuity Mycoplasma Quant Kit Handbook* "For the detection of mycoplasma using the QIAcuity dPCR System with the Spike-In Internal Control during Sample Preparation".

Important points before starting

- See "Important Notes" on page 13.
- The Pharmacopeia (EP, USP, and JP) compliant mycoplasma testing requires a nucleic acid purification before PCR, which needs to be validated as part of the workflow. QIAGEN offers a validation report available for download on the product page to demonstrate the performance of the mycoplasma testing workflow using the EZ1&2 Virus Mini Kit v2.0 (48) (QIAGEN GmbH, cat. no. 955134) and the Venor Mycoplasma Extraction (Minerva Biolabs GmbH, Berlin, cat. nos. 56-3010 and 56-3100) for nucleic acid purification in combination with the QIAcuity Mycoplasma Quant Kit for RT-dPCR.
- The validation of the mycoplasma test procedure needs to be performed using the sample matrix in which samples will be tested to show the procedure is functional with the respective sample matrix.
- As performance control of the procedure (nucleic acid purification, reverse transcription, and digital PCR) an internal control spike-in is required. To avoid degradation of the internal control by RNases within the sample matrix, the internal control has to be spiked-in together with the lysis buffer or after addition of the lysis buffer.

Things to do before starting

- Have an appropriate nucleic acid purification method ready to use. QIAGEN recommends the EZ1&2 Virus Mini Kit v2.0 (48) for automated nucleic acid purification or the Venor Mycoplasma Extraction Kit (Minerva Biolabs GmbH) that allows for manual or automated nucleic acid purification. Alternative solutions can be used after performance assessment. Be sure to choose a solution that isolates total nucleic acid from the sample.
- Prepare all components of the nucleic acid purification method according to the respective protocol, including preheating of potentially required heat blocks.
- Prepare all buffers and reagents of an appropriate nucleic acid purification method according to the respective protocol before starting the procedure.
- For an overall process control, reconstitute the lyophilized QIAcuity Mycoplasma Internal Control, briefly centrifuge the tubes to remove any material from the lid. To end up with a 10-fold higher concentrated Internal Control than for RT-dPCR only, dissolve lyophilized QIAcuity Mycoplasma Internal Control (720,000 copies) by adding 120 µL RNase-free Water to yield a final concentration of 6000 copies/µL. Incubate 5 min at ambient temperature (18–25°C), then vortex and spin the reconstituted reagents.

  Note: QIAcuity Mycoplasma Internal Control can be stored at –15°C to –30°C or at 2–8°C for up to 8 months. Avoid more than 5 freeze–thaw cycles.
- Reconstitute lyophilized QIAcuity Mycoplasma Positive Control: Briefly centrifuge the tubes to remove any material from the lid. Dissolve lyophilized QIAcuity Mycoplasma Positive Control (80,000 copies) by adding 400 µL RNase-free Water to reach a final concentration of 200 copies/µL. Incubate for 5 min at ambient temperature, and then vortex and spin the reconstituted reagents.

  Note: QIAcuity Mycoplasma Positive Control can be stored at –15°C to –30°C or at 2–8°C for up to 8 months. Avoid more than 5 freeze–thaw cycles.

- Thaw all kit components except the 100× QIAcuity OneStep Advanced Reverse Transcription Mix and mix all components (also components stored at ambient temperature and on ice) right before use.
- Place the 100× QIAcuity OneStep Advanced Reverse Transcription Mix on ice.

Procedure

1. Briefly centrifuge the tubes to remove any material from the lid. Dissolve lyophilized QIAcuity Mycoplasma Standard CFU (10 CFU) by adding 1000 µL sample matrix to reach a final concentration of 10 CFU/mL.
2. Prepare a Lysis–Buffer–IC Mix: Add 2.5 µL of the reconstituted QIAcuity Mycoplasma Internal Control to the required volume lysis buffer per sample (according to the protocol for the nucleic acid purification method). Adjust accordingly for the amount of processed samples.

   Note: As an example, using the recommended EZ1&2 Virus Mini Kit v2.0 Kit, add 2.5 µL reconstituted QIAcuity Mycoplasma Internal Control to the Buffer AVE and Carrier RNA. The prepared Buffer AVE–IC Mix is subsequently added to the lysate. For the Venor Mycoplasma Extraction Kit, add 2.5 µL reconstituted QIAcuity Mycoplasma Internal Control to the needed 250 µL lysis buffer per sample to be processed. Mix carefully by inverting for at least 15 times to avoid foam formation. Foam formation does not affect lysis performance but make sure to pipette liquid and not foam.
3. Follow the nucleic acid purification protocol using the reconstituted QIAcuity Mycoplasma Standard CFU as sample until the addition of lysis buffer. Here, the IC needs to be spiked-in for an overall process control. Two options for spiking-in the internal control are appropriate.

   a. Add the appropriate volume of prepared Lysis–Buffer–IC Mix to the sample. Vortex for 2 s and spin down.

Important: If foam formation took place, make sure to pipette liquid and not foam.

Note: The appropriate volume of lysis buffer is defined by the protocol of the nucleic acid purification method.

b. Add the appropriate volume of lysis buffer. Vortex for 2 s and spin down. Add 2.5 µL QIAcuity Mycoplasma Internal Control to the sample. Vortex for 2 s and spin down.

Note: The appropriate volume lysis buffer is defined by the protocol of the nucleic acid purification method.

4. Proceed with the nucleic acid purification protocol. It is recommended to elute into DNA low binding tubes. Directly proceed with step 5 of this protocol.

Long-term storage of eluates is not recommended.

Important: If a bead-based system is used for nucleic acid purification, it is important to avoid bead carry-over into the eluate. Beads in the RT-dPCR can negatively affect the system performance.

5. Prepare the RT-dPCR reaction mix in a standard PCR plate using the QIAcuity Mycoplasma Quant Kit according to Table 4.

6. Add the eluate obtained from the sample preparation. We recommend to add the maximum volume of 22.6 µL to the reaction, but this can be adjusted.

Note: You can add 10 µL of the reconstituted QIAcuity Mycoplasma Positive Control instead of the eluate to one or more wells to check the reaction performance. Adjust the volume by adding RNase-free-Water accordingly.

Table 4. PCR setup

Component	Volume in Nanoplate 26k (8-well and 24-well)	Final concentration in reaction
4× OneStep Adv. Probe Master Mix	10 µL	1×
100× OneStep Adv. RT Mix	0.4 µL	1×
OneStep Enhancer GC	5 µL	–
20× QIAcuity Mycoplasma Assay	2 µL	1×
QIAcuity Mycoplasma Internal Control	–*	Venor Prep: 94 copies/µL† EZ2 Prep: 104 copies/µL†
Sample‡ or QIAcuity Mycoplasma Positive Control	Up to 22.6 µL sample or 10 µL QIAcuity Mycoplasma Positive Control	50 copies/µL§
RNase-free Water	Fill up to 40 µL	–
Total reaction volume	**40 µL**	**–**

* The QIAcuity Mycoplasma Internal Control is added during the lysis step of nucleic acid purification; hence, no addition during RT-PCR setup is needed.

† The final concentration of QIAcuity Mycoplasma Internal Control depends on the elution volume of the nucleic acid purification and the sample volume analyzed in the RT-PCR. The final concentration can be calculated using the formula

$$C_{\mathrm{IC}} = \frac{V_{\text{eluate in dPCR}}}{V_{\text{total dPCR}}} \times \frac{V_{\text{IC stock}} \times C_{\text{IC stock}} \times \frac{V_{\text{IC added to lysate}}}{V_{\text{IC total}}}}{V_{\text{eluate}}}$$

where C_{IC} is the IC concentration in dPCR, $V_{\text{eluate in dPCR}}$ is the volume of eluate added to the reaction, $V_{\text{total dPCR}}$ is the total volume of dPCR, $V_{\text{IC stock}}$ is the volume of IC stock added to the lysis buffer, $C_{\text{IC stock}}$ is the concentration (in copies per microliter) of IC stock, $V_{\text{IC added to lysate}}$ is the volume of IC added to the lysate, $V_{\text{IC total}}$ is the total volume of IC prepared, and V_{eluate} is the final volume of the eluate. All volumes are in µL.

For example, according to the recommended nucleic acid purification with Venor Prep, the IC concentration in dPCR is

$$C_{\mathrm{IC}} = \frac{20}{40} \times \frac{2.5 \times 6000 \times \frac{2.5}{2.5}}{80} = 93.75 \text{ copies}/\mu\text{L}.$$

For EZ2 prep, the IC concentration in dPCR is

$$C_{\mathrm{IC}} = \frac{20}{40} \times \frac{2.5 \times 6000 \times \frac{50}{60}}{60} = 104.17 \text{ copies}/\mu\text{L}.$$

‡ Sample extract should be generated using an appropriate total nucleic acid purification technique. For recommendations, see "Things to do before starting" section of this protocol.

§ Exemplary volume of the QIAcuity Mycoplasma Positive Control. If 10 µL QIAcuity Mycoplasma Positive Control is added, a final concentration of 50 copies/µL is expected. The final concentration can be calculated using the formula

$$C_{\mathrm{PC}} = \frac{C_{\mathrm{start}} \times V_{\mathrm{PC}}}{V_{\mathrm{total}}}$$

where C_{PC} is the PC concentration in dPCR, C_{start} is the starting concentration, V_{PC} is the PC volume, and V_{total} is the total reaction volume.

7. Transfer the content of each well to a 26k nanoplate avoiding bubbles. Seal the nanoplate and load it into the QIAcuity instrument. Start the run.

Additional information regarding PCR setup, recommended cycling, and imaging conditions can be found in “Protocol: Detection of Mycoplasma Using the QIAcuity Mycoplasma Quant Kit with the Internal Control as RT-PCR Control” on page 14.

Troubleshooting Guide

The troubleshooting guidance provided here applies to general assay performance. Troubleshooting related to validated or pharmacopeia compliant workflows is described in the corresponding validation handbook.

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, visit **www.qiagen.com/FAQ/FAQList.aspx** for Frequently Asked Questions at our Technical Support Center (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
RNA purification	
Insufficient mycoplasma lysis	**Suboptimal buffer conditions for efficient mycoplasma lysis**: This is possibly due to high viscosity of the Lysis Buffer. Make sure to pipette indicated volume without air bubbles. **Incubation within lysis step skipped**: Any incubation step described in the RNA purification protocol is mandatory. Lysis performed at different temperature or for a different amount of time can lead to a reduction in mycoplasma yield and, as a consequence, to misleading test results.
Bead carry-over	**Not all beads are bound by the magnet**: Make sure the tube is placed properly in the magnetic rack to enable optimal bead binding. It is required to place the tubes for indicated period of time in the magnetic rack to enable optimal bead binding.
Sample storage after purification	**Underquantification of the mycoplasma concentration**: Sample storage after RNA extraction is possible, even though, direct processing of eluates is strongly recommended. If direct processing is not possible, RNA containing eluates should be stored at –80°C. Storage at higher temperatures can lead to degradation of RNA.

	Comments and suggestions
PCR	
Lower mycoplasma concentration than expected	**Insufficient nucleic acid extraction**: Make sure that the used extraction method is suitable for total nucleic acid extraction and follow the instructions of sample extraction as described in the respective protocol. **Nucleic acid degradation during processing**: Perform mycoplasma testing in an RNase-free and DNase-free environment. **Loss of nucleic acid during storage**: Use RNase-free and DNase-free consumables and low binding tubes. If storage cannot be avoided, eluates need to be stored at –80°C until analyzed. Repeated freeze–thaw cycles should be avoided.
Lower Internal Control concentration than expected	**Insufficient rehydration**: Add the appropriate volume of RNase-free Water to QIAcuity Mycoplasma Internal Control. **Insufficient nucleic acid extraction**: Make sure that the extraction method used is suitable for total nucleic acid extraction and follow the instructions of sample extraction as described in the respective protocols.
No positive partitions	**Sample input below LOD**. Increase sample input into RT-PCR. It is recommend to load 22.6 µL sample. **Cycling conditions**: Check and optimize annealing/extension temperature with custom designed assays or assays from other suppliers. Please follow recommended cycling program when using QIAGEN assays. **Imaging setup**: Check channel choice and match with probe dyes. Any positive mycoplasma signal is detected in the green channel, while the QIAcuity Mycoplasma Internal Control is detected in the yellow channel. **Inhibition**: Make sure that the used extraction method does not carry over any PCR inhibitor. **Shortened reverse transcription**: Run the RT-PCR with an initial RT step for 40 min at 50°C. **Prolonged initial denaturation**: Run the RT-PCR using the QIAcuity OneStep Advanced Probe mix with an initial denaturation of 2 min at 95°C which is also required to inactivate the RT enzyme. Prolonged denaturation might negatively affect PCR performance.
Infinity signal or no negative partitions	**Sample dilution**: Increase dilution to fit into the dPCR concentration range.

Ordering Information

Product	Contents	Cat. no.
QIAcuity Mycoplasma Quant Kit	OneStep Advanced Probe Master Mix, OneStep Advanced RT Mic, OneStep Enhancer GC, QIAcuity Mycoplasma Assay, QIAcuity Mycoplasma Internal Control, QIAcuity Mycoplasma Positive Control, RNase-Free Water	250261
Mycoplasma arginini Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250262
Mycoplasma orale Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250263
Mycoplasma gallisepticum Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250264
Mycoplasma pneumoniae Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250265
Mycoplasma synoviae Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250266
Mycoplasma fermentans Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250267
Mycoplasma hyorhinis Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250268
Acholeplasma laidlawii Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250269
Spiroplasma citri Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250270

Product	Contents	Cat. no.
Mycoplasma salivarium Standard CFU	QIAcuity Mycoplasma 10 CFU Standard, QIAcuity Mycoplasma Negative Control	250271
QIAcuity Nanoplate 26K 24-well (10)	10 QIAcuity Nanoplates 26K with 24 wells, 11 Nanoplate Seals	250001
QIAcuity One, 5plex Instrument	One-plate digital PCR instrument for detecting up to 5 fluorescent dyes, roller, USB flash memory, and QIAcuity Software Suite: includes 1 preventive maintenance visit. One year warranty on labor, travel, and parts also included.	911020
QIAcuity Four Instrument	Four-plate digital PCR instrument for detecting up to 5 fluorescent dyes, notebook computer, barcode scanner, roller, USB flash memory, and QIAcuity Software Suite: includes installation, training, and 1 preventive maintenance visit. One year warranty on labor, travel, and parts.	911040
QIAcuity Eight Instrument	Eight-plate digital PCR instrument for detecting up to 5 fluorescent dyes, notebook computer, barcode scanner, nanoplate roller, USB flash memory, and QIAcuity Software Suite: includes installation, training, and 1 preventive maintenance visit. One year warranty on labor, travel, and parts.	911050
EZ2 Connect	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 1-year warranty on parts and labor	9003210
Related Products		
QIAcuity Nanoplate 8.5K 24-well (10)	10 QIAcuity Nanoplates 8.5K with 24 wells, 11 Nanoplate Seals	250011
QIAcuity Nanoplate 8.5K 96-well (10)	10 QIAcuity Nanoplates 8.5K with 96 wells, 11 Nanoplate Seals	250021
QIAcuity Nanoplate 26k 8-well (10)	10 QIAcuity Nanoplate 26k 8-well, 11 Nanoplate Seals	250031
Nanoplate Seals (11)	11 Nanoplate Seals	250099

Product	Contents	Cat. no.
CGT Viral Vector Lysis Kit (100)	For 100 DNase I reactions (50 µL): CGT Sample Stabilizer, CGT DNase I Buffer, DNase I, CGT Lysis Buffer, CGT Dilution Buffer, and Nuclease-free Water	250272
CGT Viral Vector Lysis Kit (1000)	For 1000 DNase I reactions (50 µL): CGT Sample Stabilizer, CGT DNase I Buffer, DNase I, CGT Lysis Buffer, CGT Dilution Buffer, and Nuclease-free Water	250273
QIAcuity Residual DNA Quantification Sizing Kits and Standards	QIAcuity *E. coli* / CHO / HEK293 resDNA Quant Master Mix (4×) lyophilized, Positive Control, Internal Control, RNase-free Water, and respective Standard Kits	250220–250225
QIAcuity Cell & Gene Therapy (CGT) dPCR Assays	For 500 × 12 µL reactions (20×): QIAGEN Cell and Gene Therapy assay for GFP; ITR2/5, Sv40 promoter, AMP resistance, or others	250230–250256
CGT Lentivirus Lysis Kit (100)	For 100 DNase I (50 µL) and Lysis reactions with Proteinase K (40 µL): Proteinase K, DNase I, CGT PBS Buffer, Buffer RDD, CGT Dilution Buffer, and Nuclease-free Water	250323
CGT Lentivirus Lysis Kit (1000)	For 1000 DNase I (50 µL) and Lysis reactions with Proteinase K (40 µL): Proteinase K, DNase I, CGT PBS Buffer, Buffer RDD, CGT Dilution Buffer, and Nuclease-free Water	250324
QIAcuity RCL Quant Kit	For 96 reactions: QIAcuity MasterMix, QN Internal Control DNA dPCR, QN IC Probe Assay, VSV-G Assay, Positive Control, RNase-free Water	250322

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Document Revision History

Date	Description
05/2026	Initial release

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