May 2026

Quick-Start Protocol

QIAcuity® Mycoplasma Quant Kit

The QIAcuity Mycoplasma Quant Kit (cat. no. 250261) consists of the OneStep Advanced Probe Master Mix, OneStep Advanced RT Mix, OneStep Enhancer GC, Mycoplasma Assay, Internal Control, and Positive Control and can be used to detect mycoplasma contamination according to the European Pharmacopeia (EP), the US Pharmacopeia (USP), and the Japanese Pharmacopeia (JP). The kit is shipped on dry ice and should be stored at –30°C to –15°C immediately upon arrival until the expiry date indicated on the labels.

Further information

- *QIAcuity User Manual*: **www.qiagen.com/HB-2717**
- *QIAcuity User Manual Extension*: **www.qiagen.com/HB-2839**
- *QIAcuity Mycoplasma Quant Kit Handbook*: **www.qiagen.com/HB-3503**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Refer to the *QIAcuity User Manual* and *QIAcuity User Manual Extension* for guidance on using the QIAcuity platform.
- At least one No Template Control (NTC) sample should be included per run to detect any RNA or DNA contamination.
- Refer to the *QIAcuity Mycoplasma Quant Kit Handbook* for guidance on EP, USP, JP compliant workflow, including sample extraction.

Procedure

Sample preparation

1. Prepare the sample with an appropriate sample prep method to extract mycoplasma total nucleic acids.

Note: To use the QIAcuity Mycoplasma Internal Control as overall control, consult "Protocol: Detection of Mycoplasma Using the QIAcuity Mycoplasma Quant Kit with the Use of the Spike-In Internal Control During Sample Preparation" in the *QIAcuity Mycoplasma Quant Kit Handbook* (HB-3503).

Rehydrating the lyophilized reagents

2. Briefly centrifuge all tubes before usage to remove material from the lid.
3. Referring to Table 1, add the appropriate volume of RNase-free Water to the lyophilized controls.
4. Incubate for 5 min at ambient temperature (18–25°C). Vortex and spin the reconstituted reagents briefly.

Table 1. Rehydrating lyophilized reagents

Component	RNase-free Water to be added (µL)	Final concentration (copies per µL)
QIAcuity Mycoplasma Internal Control to be used as spike-in Process Control	120	6000
QIAcuity Mycoplasma Positive Control	400	200

Reaction setup

1. Prepare the RT-dPCR reaction mix using the QIAcuity Mycoplasma Quant Kit according to Table 2 in a standard PCR plate.
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.
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.
5. In the QIAcuity Software Suite or on the QIAcuity instrument, under the dPCR parameters, set the cycling conditions according to Table 3.
6. Start the run.

   Note: See the *QIAcuity User Manual* for the detailed procedure.

Table 2. 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 of Positive Control
RNase-free Water	Fill up to 40 µL	–
Total reaction volume	**40 µL**	**–**

* In this case, 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

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

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

‡ Sample extract should be generated using an appropriate total nucleic acid purification technique. For recommendations, see the *QIAcuity Mycoplasma Quant Kit Handbook*.

Table 3. QIAcuity Mycoplasma Quant Kit cycling program

Step	Time	Temperature (°C)
Reverse transcription reaction		
Initial denaturation	40 min	50
RT Enzyme inactivation	2 min	95
Two-step cycling (40 cycles)		
Denaturation	15 s	95
Annealing or elongation	1 min	59

Table 4. Recommended QIAcuity Mycoplasma Quant Kit imaging settings

Target	Detection channel	Exposure/gain
Target assay (mycoplasma)	Green	500/6
Internal control	Yellow	500/6

Document Revision History

Date	Description
10/2023	Initial release
12/2023	Sample isolation procedure for RNA and DNA being a recommended step added as a note in sample preparation procedure.
05/2026	Deleted the table listing kit contents. Minor editorial changes.

Scan the QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.