December 2023

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 to ensure best performance in detecting any mycoplasma contamination with 127 different mycoplasma species 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**

**Table 1. Kit contents**

| Component | Quantity | Cap color |
|---|---|---|
| 4x OneStep Advanced Probe Master Mix, 1 mL | 1 | Red |
| 100x OneStep Advanced RT Mix, 0.045 mL | 1 | Purple |
| OneStep Enhancer GC, 1 mL | 1 | Yellow |
| 20x QIAcuity Mycoplasma Assay, 0.2 mL | 1 | Blue |
| QIAcuity Mycoplasma Internal Control, lyophilized | 2 | Orange |
| QIAcuity Mycoplasma Positive Control, lyophilized | 1 | Green |
| RNase-Free Water, 1.9 mL | 2 | Neutral |

## Notes before starting

- Refer to the *QIAcuity User Manual* and *QIAcuity User Manual Extension* for guidance on best practices with 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 RNA.
   **Note**: We recommend our optimized protocol described in *QIAcuity Mycoplasma Quant Kit Handbook*. We also recommend performing sample isolation for RNA and DNA.
   **Note**: To use the QIAcuity Mycoplasma Internal Control as overall control, please refer to the following protocol in the *QIAcuity Mycoplasma Quant Kit Handbook*: "Detection of Mycoplasma Using the QIAcuity Mycoplasma Quant Kit with the Use of the Spike-In Internal Control During Sample Preparation".

### Rehydrating the lyophilized reagents

2. Briefly centrifuge all tubes before usage to remove material from the lid.
3. Referring to Table 2, add the appropriate volume of RNase-Free Water to the lyophilized controls.
4. Incubate for 5 min at ambient temperature (15–25°C). Vortex and spin the reconstituted reagents briefly.

**Table 2. Instructions for rehydrating lyophilized reagents**

| Component | Liquid to be added | Final concentration |
|---|---|---|
| QIAcuity Mycoplasma Internal Control to be used as Spike-In Control | 120 µL RNase-Free Water | 6000 copies/µL |
| QIAcuity Mycoplasma Positive Control | 400 µL RNase-Free Water | 200 copies/µL |

## Reaction setup

1. Prepare the RT-dPCR reaction mix using the QIAcuity Mycoplasma Quant Kit according to Table 3 in a standard PCR plate.

**Table 3. Preparing the QIAcuity Mycoplasma Quant reaction mix**

| Component | Volume/reaction Nanoplate 26k | Final concentration |
|---|---|---|
| 4x OneStep Advanced Probe Master Mix | 10 µL | 1x |
| 100x OneStep Advanced RT Mix | 0.4 µL | 1x |
| OneStep Enhancer GC | 5 µL | – |
| 20x QIAcuity Mycoplasma Assay | 2 µL | 1x |
| QIAcuity Mycoplasma Internal Control | –* | 93.75 copies/µL† |
| Sample‡ or QIAcuity Mycoplasma Positive Control | Up to 22.6 µL sample or 10 µL QIAcuity Mycoplasma Positive Control | 50 copies/µL Positive Control |
| RNase-Free Water | Fill up to 40 µL | – |
| **Total reaction volume** | **40 µL** | – |

* QIAcuity Mycoplasma Internal Control is added in 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 reaction. The final concentration can be calculated using the formula:

IC concentration in RT-dPCR = (IC spike-in vol. x IC starting conc. / elution vol.) x eluate vol. in RT-dPCR / total reaction vol.

Example: According to the recommended nucleic acid purification:

IC concentration in RT-dPCR = (2.5 µL x 6000 copies/µL / 80 µL) x 20 µL / 40 µL = 93.75 copies/µL.

‡ Sample extract should be generated using an appropriate RNA purification technique. For recommendation see handbook.

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 addition of water accordingly.

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.
5. In the QIAcuity Software Suite or on the QIAcuity instrument, under the dPCR parameters, set the cycling conditions according to Table 4.

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

**Table 4. QIAcuity Mycoplasma Quant dPCR cycling program**

| Step | Time | Temperature (°C) |
|---|---|---|
| **Reverse Transcription reaction** | | |
| Reverse transcription | 40 min | 50 |
| RT Enzyme inactivation | 2 min | 95 |
| **Two-step cycling (40 cycles)** | | |
| Denaturation | 15 s | 95 |
| Annealing / Elongation | 1 min | 59 |

**Table 5. Recommended QIAcuity Mycoplasma Quant dPCR imaging settings**

| Target | Detection channel | Exposure/gain |
|---|---|---|
| Target assay | 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. |

Scan QR code for *QIAcuity Mycoplasma Quant Kit Handbook*.

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