

Quick-Start Protocol

EZ1&2® Virus Mini Kit v2.0 - Mycoplasma

Mycoplasma extraction using the EZ2® Connect instruments

For usage in other applications than mycoplasma nucleic acid extraction using the EZ1&2 Virus Mini Kit v2.0 with EZ2 instruments, please refer to the respective Handbook (www.qiagen.com/HB-2978) and Quick-Start Protocol (www.qiagen.com/HB-2977).

For the usage with EZ1 $^{\circledR}$ instruments, please refer to the respective Handbook (www.qiagen.com/HB-0113) and Quick-Start Protocol (www.qiagen.com/HB-0787). The EZ1&2 Virus Mini Kit v2.0 (cat. no. 955134) can be stored at ambient temperature (15–25 $^{\circ}$ C) until the indicated expiration date. Do not freeze the reagent cartridges.

Further information

- EZ1&2 Virus Handbook: www.qiagen.com/HB-2978
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com
- QIAcuity Mycoplasma Quant Kit Handbook: www.qiagen.com/HB-3503

Notes before starting

- It is recommended to use the Mycoplasma Internal Control from the QIAcuity Mycoplasma Quant Kit (cat. no. 250261) as workflow control by spiking-in the Mycoplasma Internal Control during the mycoplasma nucleic acid extraction.
- The buffer in well 1 of the reagent cartridge may form a precipitate upon storage. If necessary, re-dissolve by warming at 37°C and then place at ambient temperature (15–25°C).
- Before loading reagent cartridges into the EZ2 Connect instrument, invert the cartridges
 four times to mix the magnetic particles, and then tap to deposit the reagents at the bottom
 of their wells. Check that the magnetic particles are completely resuspended.

Procedure

- 1. Reconstitute Carrier RNA (310 μg) in 310 μL RNase-Free Water to gain a final concentration of 1 $\mu g/\mu L$.
- 2. Reconstitute Mycoplasma Internal Control in 120 μ L RNase-Free Water to gain a final concentration of 6000 copies/ μ L.
- Prepare Carrier RNA Internal Control by mixing 1 μL Carrier RNA (1 μg/μL),
 2.5 μL Mycoplasma Internal Control (6000 cp/μL), and 56.5 μL AVE Buffer in a 1.5 mL tube (provided).
- 4. Add 400 μL of your sample to a 2.0 mL tube (provided). If less than 400 μL of sample is available, fill the volume up to 400 μL with Buffer AVE.
- 5. Turn on the EZ2 Connect instrument.
- 6. Tap Virus on the Applications panel, select Virus Mini Kit v2.0, and press Next.
- 7. Choose the Mycoplasma protocol. Press Next.
- 8. Choose "400 μL sample" and "60 μL elution volume". Press Next.
- 9. Select the positions on the work deck according to the number of samples to be processed. Press **Next**.
- 10. Enter sample IDs or press **Generate missing sample IDs**. Then press **Next**.

- 11. Load the EZ1&2 Virus reagent cartridges into respective positions of the EZ2 Connect Cartridge Rack, as selected in step 10.
- 12. Place empty non-skirted 2 mL tubes (provided) into position 11 of the cartridges.
- 13. Open the instrument hood. Load the EZ2 Connect Cartridge Rack into the EZ2 Connect instrument
- 14. Remove caps of all tubes and prepare the EZ2 Connect Tip Rack as follows:
 - ° Position A: 2.0 mL tube, containing 400 µL sample
 - Position B: 1.5 mL tube with Carrier RNA Internal Control mix
 - Position C: Tip holder with Filter Tip
 - Position D: 1.5 mL empty tube

Press **Next**.

- 15. Place the EZ2 Connect Tip Rack into the EZ2 Connect instrument and start the run according to the instructions on the instrument display.
- 16. The display will show "Protocol finished" when the run is completed. Select **Finish**.
- 17. Open the instrument hood. Remove the elution tube containing purified nucleic acid from position D of the EZ2 Connect Tip Rack. Discard the used cartridge including the liquid waste.

Optional: Follow onscreen instructions for UV decontamination of worktable surfaces.

18. Perform regular maintenance after each run. Press **Finish** to return to the home screen.

Document Revision History

Date	Changes
02/2025	Initial release.

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