Quick-Start Protocol June 2018

MagAttract® PowerMicrobiome® DNA/RNA EP Kit

All reagents and kit components of the MagAttract PowerMicrobiome DNA/RNA EP Kit can be stored at room temperature (15–25°C).

Further information

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

Notes before starting

- Warm the Lysis Solution MBL at 60°C for 10 min before use to dissolve precipitates.
- Add β-mercaptoethanol (β-ME) at a ratio of 25 µl per ml of the Solution MBL. You will need 64 ml of Solution MBL/β-ME per 96 well plate.

1. Centrifuge PowerBead DNA Plate, Glass 1.0 mm at 4500 x *g* for 1 min. Carefully peel off the Elution Sealing Mat that covers the PowerBead DNA Plate and discard.
2. Add 650 µl of warmed Solution MBL/β-ME to each well of the PowerBead DNA Plate. **Optional:** Enhance recovery and integrity of RNA by adding 100 µl of phenol:chloroform:isoamyl alcohol (PCI) (25:24:1, pH 6.5–8) to the wells of the bead plate pre-loaded with 650 µl of Solution MBL/β-ME before filling with stool samples.
3. Add 0.25 g of sample to each well of the PowerBead DNA Plate.
4. Secure a 2 ml Sealing Mat tightly to the PowerBead DNA Plate, sealing well. Vortex horizontally for 5 s on the vortex ensuring the solution/sample is well mixed. **Note:** If needed, this is a stopping point. You can store the PowerBead DNA Plate at –15 to –30°C covered with a new Sealing Mat.
5. Place each PowerBead DNA Plate (with Sealing Mat securely affixed) between 2 Adapter Plates (cat. no. 11990). Place on the TissueLyzer II (cat. no. 85300). Shake at speed 20 for 10 min.
6. After the first 10 min cycle, remove the block and rotate it so that the side closest to the machine body is now furthest from the machine. Shake again at speed 20 for 10 min.
7. Centrifuge the PowerBead DNA Plate at room temperature at 4500 x *g* for 6 min.

8. Carefully, without splashing, remove and discard the Sealing Mat and transfer the supernatant to a clean 2 ml Collection Plate.
   Note: The supernatant may still contain some biosolid particles.
9. Add 150 µl of Solution IRS to each well and apply Sealing Tape to the 2 ml Collection Plate. Vortex horizontally for 5 s until solution is well mixed. Incubate at 2–8°C for 5 min.
10. Centrifuge the Collection Plate at room temperature for 6 min at 4500 x *g*. Remove and discard Sealing Tape.
11. Avoiding the pellet, transfer the entire volume of supernatant to a new 2 ml Collection Plate. Apply Sealing Tape to the 2 ml Collection Tube. Centrifuge again at 4500 x *g* for 6 min to clear any residual particulates that may have carried over.
12. Transfer no more than 850 µl of supernatant to a new 2 ml Collection Plate, again avoiding any residual pellet.
    Note: You may place the supernatant in the 2 ml Collection Plate at 2–8 °C for several hours if you need to stop or if you can only process one 96 well plate at a time.
13. Place the 2 ml Collection Plate containing the supernatant on the epMotion® robotic deck as shown in the epMotion program.
14. For each 96 well plate to be processed, place 174 ml of ClearMag® Wash Solution into an Eppendorf 400 ml reservoir set according to the worktable in the epMotion program.
15. For each 96 well plate to be processed, place 11 ml of RNase-free water (provided) into an Eppendorf 30 ml reservoir placed in an Eppendorf tub holder located as indicated on the worktable.
16. For each 96 well plate to be processed, prepare the ClearMag Binding Solution/ClearMag Beads (Zorb Reagent) by first vortexing the bottle containing the ClearMag Beads until all beads are resuspended, followed by adding 2 ml of the ClearMag Beads to 85 ml of the ClearMag Binding Solution. Vortex well to mix.
17. Transfer the entire volume of ClearMag Binding Solution/ClearMag Beads into an Eppendorf 100 ml reservoir in an Eppendorf tube holder as indicated on the worktable.
18. Initiate the protocol.
    Note: Start the protocol immediately otherwise the beads will begin to settle. If there is a delay (in excess of 3 min) then re-agitate the beads.
19. Upon completion, cover the wells of the 96 well Microplate with the Elution Sealing Mat (provided). DNA is now ready for any downstream application. We recommend storing DNA frozen (–20°C or –80°C).

Revision history

Document	Date	Description of changes
MagAttract PowerMicrobiome DNA/RNA EP Kit Quick-Start Protocol	March 2017	Initial release
MagAttract PowerMicrobiome DNA/RNA EP Kit Quick-Start Protocol	September 2017	Updated storage information
MagAttract PowerMicrobiome DNA/RNA EP Kit Quick-Start Protocol	June 2018	Added QR code, updated dates, added revision history