Quick-Start Protocol June 2018

MagAttract® PowerMicrobiome® DNA/RNA KF Kit

All reagents and kit components of the MagAttract PowerMicrobiome DNA/RNA KF 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 Lysis Solution MBL at 60°C for 10 min before use. Use while still warm.
- 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 the PowerBead DNA Plate, Glass 0.1 mm at 4500 x *g* for 1 min to bring beads down into the well. 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 grams 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 of the PowerBead DNA Plates (with Sealing Mats securely affixed) between 2 Adapter Plates (cat. no. 11990) and 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. Shake again for 10 more min at speed 20.
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 1 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 1 ml Collection Plate. Vortex horizontally for 5 s until solution is well mixed. Incubate at 2–8°C for 5 min.
10. Centrifuge the 1 ml 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 1 ml Collection Plate. Apply Sealing Tape to the 1 ml Collection Plate. Centrifuge again at 4500 x *g* for 6 min to clear any residual particulates that may have carried over.
12. Transfer no more than 450 µl of supernatant from each well to the wells on a clean KingFisher® Deep Well 96 Plate.
    Note: Lysate can be stored at 2–8°C for several hours if processing cannot continue immediately in clean KingFisher Deep Well 96 Plates.
13. Proceed based on the protocols specific to your instrument. For the KingFisher Duo Protocol, please refer to the respective section of the handbook.

KingFisher Flex protocol

14. For each 96 well plate to be processed, resuspend the ClearMag® Beads (Zorb Reagent) by vortexing the bottle and add 2 ml of the resuspended ClearMag Beads to 45 ml of the ClearMag Binding Solution in an appropriate vessel. Mix well. Immediately transfer to a multichannel reservoir.
    Note: The ClearMag Beads/ClearMag Binding Solution will slowly settle. Maintain the beads in suspension for uniform distribution in the next step.
15. Add 470 µl of the ClearMag Beads/ClearMag Binding Solution to each well of lysate in a KingFiser Microtiter Deep Well 96 Plate.
16. Place the KingFisher Microtiter Deep Well 96 Plate containing the lysate and ClearMag Beds/ClearMag Binding Solution onto the robotic deck as indicated in the program.
17. Place 500 µl of ClearMag Wash Solution into each well of the three clean KingFisher Microtiter Deep Well 96 plates and place on the deck as indicated in the program.
18. Place 100 µl of RNase-free water (provided) into each well of a KingFisher 96 KF plate and place on the deck at the specified location. Initiate the KingFisher MO BIO PowerMag® Microbiome robotic program the protocol.
19. Upon completion, cover the wells of the KingFisher 96 KF plate with an appropriate storage seal. DNA and RNA are 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 KF Kit Quick-Start Protocol	March 2017	Initial release
MagAttract PowerMicrobiome DNA/RNA KF Kit Quick-Start Protocol	September 2017	Updated storage information, harmonized temperature indications, added QR code
MagAttract PowerMicrobiome DNA/RNA KF Kit Quick-Start Protocol	June 2018	Added revision history