Quick-Start Protocol February 2017

MagAttract® Microbial DNA Kit (384)

RNase A Solution should be stored at 2–8°C. All other reagents and kit components of the MagAttract Microbial DNA Kit (384) 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 minutes before use. Use while still warm.
- Before starting, add 9 µl of the provided RNase A Solution per 1 ml of warmed Solution MBL. To allow for pipetting variations and overage for the reagent reservoir, add 315 µl of the RNase A Solution to 35 ml of Solution MBL. For KingFisher® Duo applications add 350 µl of Solution MBL followed by 3 µl of RNase A Solution.
- You will need 333 ml of 100% ethanol for each full 96 well plate processed on the KingFisher and 363 ml of 100% ethanol for the epMotion®. The KingFisher Duo requires 36 ml for each 12 wells processed.
- Before first use, centrifuge bead plates at 4500 x *g* for 3 min. Remove and discard mat.

1. Dispense 1.8 ml of liquid culture into each well of the 2 ml Collection Plate, and cover with Sealing Tape. Centrifuge at 4500 x *g* for 12 min.
2. Discard tape and remove media without disturbing cell pellet. Excessive amounts of residual media will dilute the lysis chemistry, only minimal amounts of residual media should be in each well.
3. Add 350 µl of Solution MBL/RNase A and apply fresh Sealing Tape (provided). Resuspend the cell pellet completely by high speed vortexing. Centrifuge briefly to ensure that the cell suspension is at the bottom of the wells. Avoid repelleting the cells.
4. Centrifuge PowerBead DNA Plates, Glass 0.1 mm at 4500 x *g* for 3 min. Remove Elution Sealing Mat and discard. Transfer cell suspension into PowerBead DNA Plates.
5. Seal PowerBead DNA Plates with new Sealing Mats .
6. Place each PowerBead DNA Plate (with Sealing Mat securely affixed) between 2 Adapter Plates (cat. no. 11990) Place on a TissueLyzer II (cat.

no. 85300); Refer to the protocol provided for placement of Adapter Plates. Shake 5 min at speed 20.

7. Remove plates and re-orient them so that the side closest to the machine body is now furthest from the machine body and shake again at speed 20 for 5 minutes.
8. Centrifuge the PowerBead DNA Plates at 4500 x *g* for 6 min.
9. Remove and discard Sealing Mat. Avoiding the glass beads, transfer the supernatant to a clean 1 ml Collection Plate.
10. Add 100 µl of Solution IRS to the wells of the plate and cover with Sealing Tape. Vortex for 5 s. Incubate at 2–8 °C for 10 min, then centrifuge the plate at 4500 x *g* for 9 min.
11. Open the protocol specific to your platform. For epMotion and KingFisher Duo please refer to the respective section in the Handbook.

KingFisher Flex protocol

12. Avoiding the pellet, transfer up to 450 µl of supernatant from Step 10 to the appropriate wells on a KingFisher Microtiter Deep Well 96 Plate.
    Note: Supernatant may stay in the plate at 2–8°C for several hours if you need to stop during the protocol or if you can only process one 96 well plate at a time.
13. Resuspend the SwiftMag® Beads by vortexing. Add 5 ml of the resuspended SwiftMag Beads to 45 ml of 100% ethanol in an appropriate vessel per plate. Immediately transfer to a multi-channel reservoir.
14. Mix well. Add 500 µl of the SwiftMag Beads/100% ethanol to each well with lysate.
    Note: Work quickly. Maintain beads in suspension for uniform distribution to each well.
15. Place the KingFisher Microtiter Deep Well 96 Plate containing the lysate/ SwiftMag Beads and ethanol on the deck as indicated on the instrument display.
16. You will need three KingFisher Microtiter Deep Well 96 Plates for the next step. Add 1 ml of 100% ethanol into each well of three KingFisher Microtiter Deep Well 96 Plates and place on the deck as indicated in the display.
17. Place 100 µl of the Solution EB into each corresponding well of a KingFisher 96 KF plate and place on the deck as indicated.
18. Initiate the KingFisher MO BIO PowerMag® Microbial DNA Isolation protocol program.
19. Upon completion, cover the wells of the KingFisher 96 KF plate with an appropriate storage seal. DNA is now ready for downstream applications. We recommend storing DNA frozen (–20°C or –80°C). Solution EB contains no EDTA.