Quick-Start Protocol November 2016

DNeasy® PowerBiofilm® Kit

The DNeasy PowerBiofilm Kit can be stored at room temperature (15–25°C) until the expiry date printed on the box label.

Further information

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

Notes before starting

- Warm Solution MBL at 55°C for 5–10 min to dissolve precipitates prior to each use. Solution MBL should be used while still warm.
- If Solution MR has precipitated, warm at 55°C for 5–10 minutes.
- Shake to mix Solution PW before use.
- Use only PowerBiofilm Bead Tubes with this kit.

1. Weigh out 0.05–0.20 g of biofilm material and place into a 2 ml collection tube (provided). Centrifuge at 13,000 x *g* for 1 min. Remove excess liquid using a pipette tip. **Note:** Add less saturated samples (e.g. microbial mats) directly to the PowerBiofilm Bead Tube (for information on selecting the right amount of starting material, refer to the Troubleshooting Guide).
2. Resuspend the biofilm material in 350 µl of Solution MBL and transfer to the PowerBiofilm Bead Tube. For less saturated samples, add 350 µl of Solution MBL to the PowerBiofilm Bead Tube already containing the biofilm material.
3. Add 100 µl of Solution FB. Vortex briefly to mix.
4. Incubate the PowerBiofilm Bead Tube at 65°C for 5 min.
5. Bead beat the sample following one of the methods described below:

   a) PowerLyzer® 24 Homogenizer

   1. Identify each PowerBiofilm Bead Tube on **both** the cap and on the side.
   2. Properly balance the Bead Tubes in the tube holder of the PowerLyzer 24. Homogenize for 1 cycle at 3200 rpm for 30 s.

3. Centrifuge the tube at 13,000 x *g* for 1 min. Transfer the supernatant to a new 2 ml collection tube (provided).
Note: Expect approximately 325–400 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

b) Vortex Adapter

1. Secure the PowerBiofilm Bead Tube horizontally to a Vortex Adapter and vortex at maximum speed for 10 min.
Note: If using the 24 place Vortex Adapter for ≥12 preps, increase time by 5–10 min.
2. Centrifuge the tube at 13,000 x *g* for 1 min at room temperature. Transfer the supernatant to a clean 2 ml collection tube (provided).
Note: Expect approximately 400–450 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

6. Add 100 µl of Solution IRS and vortex briefly to mix. Incubate at 4°C for 5 min.
Note: Use 200 µl of Solution IRS if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. Refer to the Troubleshooting Guide.
7. Centrifuge the tube at 13,000 x *g* for 1 min at room temperature.
8. Avoiding the pellet, transfer all of the supernatant to a 2 ml collection tube (provided).
Note: Expect approximately 375–450 µl in volume depending on sample material.
9. Add 900 µl of Solution MR and vortex briefly to mix.
10. Load 650 µl of supernatant onto a MB Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and repeat until all the supernatant has been processed
11. Place the MB Spin Column into a clean 2 ml collection tube (provided).
12. Add 650 µl of Solution PW and centrifuge at 13,000 x *g* for 1 min at room temperature.
13. Discard the flow-through and add 650 µl of ethanol (provided) and centrifuge at 13,000 x *g* for 1 min at room temperature.
14. Discard the flow-through and centrifuge again at 13,000 x *g* for 2 min.
15. Place the MB Spin Column basket into a clean 2 ml collection tube (provided).
16. Add 100 µl of Solution EB to the center of the white filter membrane.
17. Centrifuge at 13,000 x *g* for 1 min.
18. Discard the MB Spin Column. The DNA is now ready for downstream applications.