Quick-Start Protocol November 2016

RNeasy® PowerBiofilm® Kit

Remove lyophilized DNase I and store at 2–8°C upon arrival. DNase I should be stored at 4°C when lyophilized and –20°C after resuspension (Do not vortex the resuspended DNase; it is sensitive to physical denaturation). All other components of the RNeasy 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.
- Shake to mix Solution PW before use.
- Use only PowerBiofilm Bead Tubes with this kit.
- Add 5 µl of β-mercaptoethanol (βME) to 345 µl of Solution MBL (i.e. a total of 350 µl) for each sample to be processed. Prepare just enough fresh Solution MBL/βME for samples to be processed that day instead of adding βME to the entire bottle of Solution MBL. Use a fume hood when using βME to avoid exposure.
- Prepare DNase I stock solution by adding 300 µl RNase-free water to the lyophilized DNase I and mixing gently. Aliquot the enzyme in 50 µl portions and store at –20°C. **Note:** The enzyme can be freeze/thawed up to three times without loss of activity. To prepare DNase I Solution, combine 5 µl of DNase I stock solution with 45 µl of DNase Digestion Solution (i.e. a total of 50 µl) for each sample to be processed.

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 1min. 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/βME and transfer to the PowerBiofilm Bead Tube.
   Note: For less-saturated samples, add 350 µl of Solution MBL/βME directly to the PowerBiofilm Bead Tube 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. Secure the PowerBiofilm Bead Tube horizontally to a Vortex Adapter.
6. Vortex at maximum speed for 10 min.
   Note: If using the 24 place Vortex Adapter for >12 preps, increase time by 5–10 min.
7. Centrifuge the tubes at a **maximum** speed of 13,000 x *g* for 1 min at room temperature.
8. 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.
9. 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.
10. Centrifuge the tubes at 13,000 x *g* for 1 min at room temperature.
11. Avoiding the pellet, transfer all the supernatant to a 2 ml Collection Tube (provided).
    Note: Expect approximately 375–450 µl in volume depending on sample material.
12. Add 450 µl of Solution PB and 450 µl of ethanol (provided) and vortex briefly to mix.
13. Load 650 µl of supernatant onto a MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 minute. Discard the flow-through and repeat until all the supernatant has been loaded onto the Spin Filter.
14. Add 650 µl of Solution PW and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through and centrifuge again at 13,000 x *g* for 1 min to remove residual wash.
15. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).
16. Add 50 µl of DNase I Solution to the center of the MB Spin Column. Incubate at room temperature for 15 min.
17. Add 400 µl of Solution WB and centrifuge the column at 13,000 x *g* for 1 min.
18. Discard flow-through. Add 650 µl of Solution PW. Centrifuge at 13,000 x *g* for 1 min.
19. Discard flow-through. Add 650 µl of ethanol and centrifuge at 13,000 x *g* for 1 min.
20. Discard flow-through. Centrifuge at 13,000 x *g* for 2 min to remove residual wash.
21. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). Add 100 µl of RNase-free water (provided) to the center of the white filter membrane.
    Note: Eluting with 100 µl of RNase-free water will maximize RNA yield. For more concentrated RNA, a **minimum** of 50 µl of RNase-free water can be used.
22. Centrifuge at 13,000 x *g* for 1 minute.
23. Discard the MB Spin Column. The RNA is now ready for downstream applications.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, Sample to Insight®, RNeasy®, PowerBiofilm® (QIAGEN Group). 1104510 11/2016 HB-2232-001 © 2016 QIAGEN, all rights reserved.