Quick-Start Protocol

Allprep® PowerViral® DNA/RNA Kit

All reagents and kit components of the Allprep PowerViral DNA/RNA Kit should 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 Solution PM1 at 55°C for 10 min prior to use to dissolve precipitates. Use Solution PM1 while still warm. Shake to mix before using.
- For RNA isolation, add β-mercaptoethanol (β-ME) to Solution PM1 to produce a Solution PM1/β-ME mixture with a final β-ME concentration of 10 μl/ml. The Solution PM1/β-ME mixture loses its effectiveness over time, so prepare a fresh batch each time you use the kit. You will need 600 μl of the Solution PM1/β-ME mixture per prep.
- Bead beating is optional but should be used when lysis of microbial cells is desired or if the starting sample contains solid material that needs dispersing. For viral nucleic acid isolation from liquid samples, bead beating is generally not required.

1. If bead beating is not required, place 200 μl of viral concentrate or water into a 2 ml Collection Tube (provided). If using bead beating, skip to Step 3.
2. Add 600 μl of the Solution PM1/β-ME mixture (see ‘Notes before starting’) to the 2 ml Collection Tube. Alternatively, you may add 600 μl of Solution PM1 and 6 μl of β-ME to the Collection Tube. Vortex the viral concentrate and the Solution PM1/β-ME mixture for 30 s. Incubate for 5 min at room temperature. Go to Step 8.
3. If bead beating is required, add either 0.25 g of stool/biosolid or 200 μl of liquid into a 0.1 mm Glass PowerBead Tube (provided).
   Note: If a phenol-based lysis is desired, add 100 μl of phenol/chloroform/isoamyl alcohol (pH 6.5–8.0) to the PowerBead Tube before adding the sample.
4. Add 600 μl of the Solution PM1/β-ME mixture to the PowerBead Tube. Alternatively, you may add 600 μl of Solution PM1 and 6 μl of β-ME to the PowerBead Tube.
5. Secure the bead tubes horizontally to a Vortex Adapter (cat. no. 13000-V1-24). The tube caps should be pointing toward the center of the Vortex Adapter.

6. Vortex at maximum speed for 10 min.

7. Centrifuge at 13,000 x g for 1 min at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided). If you added phenol/chloroform/isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube (provided).

8. Add 150 µl of Solution IRS and vortex briefly to mix. Incubate at 4°C for 5 min.

9. Centrifuge the tubes at 13,000 x g for 1 min. Avoiding the pellet, transfer the supernatant to a clean 2.2 ml Collection Tube. Do not transfer more than 700 µl.

10. Add 600 µl each of Solution PM3 and Solution PM4. Vortex briefly to mix.
    **Note:** To purify small RNAs, such as microRNAs and siRNAs, transfer the lysate to a larger tube to accommodate a higher volume (2.5 ml) and add an additional 600 µl of 100% ethanol (user provided) to the lysate.

11. Load 625 µl of supernatant onto an 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 loaded onto the Spin Column.
    **Note:** A total of three loads is required for each sample processed (four loads if an additional volume of 100% ethanol was added for the microRNA/siRNA protocol).

12. Shake to mix Solution PM5 and add 600 µl to the MB Spin Column. Centrifuge at 13,000 x g for 1 min.

13. Discard flow through. Add 600 µl of Solution PM4. Centrifuge at 13,000 x g for 1 min.

14. Discard flow through and centrifuge at 13,000 x g for 2 min.

15. Place the MB Spin Column into a clean 2 ml Collection Tube (provided).

16. Add 100 µl of RNase-free water (provided) to the center of the white column membrane. Incubate for at least 1 min.
    **Note:** Eluting with 100 µl of RNase-free water will maximize DNA/RNA yield. For more concentrated DNA/RNA, a minimum of 50 µl of RNase-free water can be used.

17. Centrifuge at 13,000 x g for 1 min. Discard the MB Spin Column. The DNA/RNA is now ready for downstream applications. RNA can be stored at −65 to −90°C.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, Sample to Insight®, AllPrep®, PowerViral® (QIAGEN Group). 1104519 02/2017 HB2241-001 © 2017 QIAGEN, all rights reserved.