Quick-Start Protocol January 2017

RNeasy® PowerPlant® Kit

The RNeasy PowerPlant 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

- Solution MBL must be warmed at 55°C for 5–10 minutes to dissolve precipitates prior to use. Use Solution MBL while still warm.
- Add the appropriate amount of β-mercaptoethanol (βME) to Solution MBL to produce a final concentration of 10 µl/ml of MBL/βME. For each prep, 600 µl of MBL/βME will be needed. Alternatively, add 594 µl of MBL and 6 µl of βME directly to each bead tube.

1. Place up to 50 mg of plant sample into a 2 ml PowerBead Tube (provided).
   Note: We recommended cutting samples into smaller pieces before weighing/loading.
2. Add 600 µl of Solution MBL/βME to the PowerBead Tube. You may prepare Solution MBL/βME in advance or add 594 µl of MBL and 6 µl of βME directly to each bead tube.
   Note: If sample is high in phenolics and you are using the Phenolic Separation Solution, reduce Solution MBL/βME to 550 µl and add 50 µl of the Phenolic Separation Solution.
3. For the highest yields of RNA, a high powered bead beater is recommended.
   A. On the PowerLyzer® 24 Instrument, we recommend a starting setting of 1 cycle at 4200 rpm for 45 s for leaf tissue and seeds.
   B. You may also homogenize using a Vortex Genie® 2 and Vortex Adapter (cat. no. 13000-V1-24) for soft leaf tissue only. Set the vortex on full speed for 10 min.
4. Centrifuge at 13,000 x *g* for 2 min at room temperature.
5. Transfer all the supernatant to a clean 2 ml collection tube (provided). Expect between 500 to 600 µl of lysate.
6. Add 150 µl of Solution IRS and vortex briefly to mix. Incubate at 4°C for 5 min.

Note: For plant samples that still contain PCR inhibitors after RNA purification, try adding up to 200 µl of Solution IRS.

7. Centrifuge the tubes at 13,000 x *g* for 2 min. Avoiding the pellet, transfer the supernatant to a clean 2 ml collection tube. Transfer no more than 650 µl at this step.
8. Add 650 µl of Solution PM3 and 650 µl of 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 the higher volume (2.6 ml), and add an additional 650 µl of 100% ethanol. You will need to supply the 100% ethanol.
9. Load 650 µl of supernatant onto an MB RNA Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow through and place the Spin Column back into the 2 ml collection tube. Repeat until all the supernatant has been loaded onto the Spin Column.
   Note: A total of three loads for each sample processed are required (four loads if an additional volume of 100% ethanol is added for the miRNA and siRNA protocol).
10. Shake to mix Solution PM5. Add 600 µl of Solution PM5 to the MB RNA Column and centrifuge at 13,000 x *g* for 1 min.
    Optional: If the RNase-Free DNase Set (cat. no. 79254-50) was purchased separately, it should be incorporated after step 10.
11. Discard the flow through, place the MB RNA Spin Column back into the 2 ml collection tube, and add 600 µl of Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
12. Discard the flow through and centrifuge again at 16,000 x *g* for 2 min.
13. Place the MB RNA Spin Filter into a clean 2 ml collection tube.
14. Add 50-100 µl of RNase-free water (provided) to the center of the white Spin Column membrane. Incubate at room temperature for 1 min.
    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.
15. Centrifuge at 13,000 x *g* for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for downstream applications and may be stored at –65° to –90°C.