Quick-Start Protocol July 2017

RNeasy® PowerSoil® Total RNA Kit

The RNeasy PowerSoil Total RNA 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

- Perform all centrifugation steps at room temperature (15–25°C).
- Wear RNase-free gloves at all times and remove RNase from the work area.

1. Add up to 2 g of soil to the 15 ml PowerBead Tube (provided). Please refer to the Troubleshooting Guide for information regarding the amount of soil to process.
2. Add 2.5 ml of PowerBead Solution, 0.25 ml of Solution SR1 and 0.8 ml of Solution IRS.
3. Add 3.5 ml of phenol/chloroform/isoamyl alcohol (pH 6.5–8.0, [User supplied]). Cap and vortex the PowerBead Tube to mix until the biphasic layer disappears.
4. Place the PowerBead Tube on a Vortex Adapter (cat. no. 13000-V1-15) and vortex at maximum speed for 15 min.
5. Remove the PowerBead Tube and centrifuge at 2500 x *g* for 10 min.
6. Transfer the upper aqueous phase (avoid the interphase and lower phenol layer) to a clean 15 ml Collection Tube (provided). Discard the phenol/chloroform/isoamyl alcohol. **Note:** The biphasic layer will be thick and firm in soils high in organic matter and may need to be pierced to remove the bottom phenol layer.
7. Add 1.5 ml of Solution SR3 to the aqueous phase and vortex to mix. Incubate at 2–8°C for 10 min and then centrifuge at 2,500 x *g* for 10 min at room temperature.
8. Transfer the supernatant, without disturbing the pellet (if there is one), to a new 15 ml Collection Tube (provided).
9. Add 5 ml of Solution SR4 to the supernatant in the Collection Tube and invert or vortex to mix. Incubate at room temperature for 30 min.

Note: Previous protocol instructions were to incubate at –20°C. If you have achieved good results for your soil type using the previous protocol, you may continue to follow it.

10. Centrifuge at 2500 x *g* for 30 min.
11. Decant the supernatant and ~~invert the 15 ml Collection Tube on a paper towel for 5 min~~.
12. Shake Solution SR5 to mix and add 1 ml to the 15 ml Collection Tube. Resuspend the pellet completely by repeatedly pipetting or vortexing.
    Note: If the pellet is difficult to resuspend, place the tube in a heat block or water bath at 45°C for 10 min, followed by vortexing. Repeat until the pellet is resuspended.
13. Prepare one JetStar Mini Column (provided) for each RNA isolation sample:
    13a. Remove the cap of a 15 ml Collection Tube (provided) and place the JetStar Mini Column inside it. The column will hang in the Collection Tube.
    13b. Add 2 ml of Solution SR5 to the JetStar Mini Column. Allow it to completely gravity flow through the column and collect in the 15 ml Collection Tube.
    Note: Do not allow the column to dry out before loading the RNA isolation sample.
14. Add the RNA isolation sample from Step 12 onto the JetStar Mini Column and allow it to gravity flow through the column into the 15 ml Collection Tube. 3ml
15. Add 1 ml of Solution SR5 to the JetStar Mini Column and allow it to completely gravity flow into the 15 ml Collection Tube. 4ml
16. Transfer the JetStar Mini Column to a new 15 ml Collection Tube (provided). Shake Solution SR6 to mix and then add 1 ml to the JetStar Mini Column to elute the bound RNA. Allow Solution SR6 to gravity flow into the 15 ml Collection Tube.
17. Transfer the eluted 4ml RNA to a 2.2 ml Collection Tube (provided). Add 1 ml of Solution SR4. Invert at least once to mix and incubate at –15°C to –30°C for a minimum of 10 min.
18. Centrifuge the 2.2 ml Collection Tube at 13,000 x *g* for 15 min to pellet the RNA.
19. Decant the supernatant and ~~invert the 2.2 ml Collection Tube onto a paper towel for 10 min to~~ air dry the pellet.
20. Resuspend the RNA pellet in 100 µl of Solution SR7.