Quick-Start Protocol February 2017

NoviPure® Soil Protein Kit (20)

Kit reagents should be stored at 2–8°C and used while cold. All other components can 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

- Pre-chill Solutions SP1, SP2 and a centrifuge to 2–8°C before starting. Shake to mix all solutions before use. All steps should be done with the samples kept on ice.
- HPLC-grade acetone should be stored at –15 to –30°C. Keep cold for all washing steps.
- Prepare a 10 ml stock solution of 1M Dithiothreitol (DTT) by adding 1.55 g of DTT to ddH_2O. Aliquot and freeze at –15 to –30°C. Each sample to be processed will require 150 µl of 1M DTT. Ensure that DTT solution is at 2–8°C during use.
- Prepare 100% (w/v) Trichloroacetic acid (TCA) by adding 227 ml of ultrapure or HPLC-grade water per 500 g of TCA. Each 1 ml of protein extract to be precipitated will require 0.25 ml of TCA. Store and use TCA solution keeping it at 2–8°C.

1. Add 5 g of soil to a 50 ml NoviPure Bead Tube (provided) and place the tube on ice.
2. Add 15 ml of cold Solution SP1 while keeping the NoviPure Bead Tube on ice.
3. Add 150 µl of 1M DTT to the NoviPure Bead Tube (final concentration of 10mM).
4. Vortex or shake to mix completely, and incubate on ice or at 2–8°C for 10 min.
5. Attach the NoviPure Bead Tube to a Vortex Adapter (cat. no.13000-V1-50) and vortex (in a cold room or refrigerator, if possible) at the highest speed for 10 min.
6. Quick spin the NoviPure Bead Tube at 4500 x *g* in a refrigerated centrifuge (2–8°C) for 30 s to ensure residual soil/beads/buffer are removed from the top of the tube and cap.
7. Place the NoviPure Bead Tube back on ice and add 1.5 ml of cold Solution SP2.
8. Vortex or shake to mix completely, and incubate on ice or at 2–8°C for 30 min.
9. Shake to mix and then attach the NoviPure Bead Tube to the Vortex Adapter and vortex (in a cold room or refrigerator, if possible) at the highest speed for 10 min.
10. Centrifuge at 4500 x *g* for 10 min in a refrigerated centrifuge (2–8°C).
11. Using a pipet, transfer supernatant to a clean, 50 ml Falcon™ Collection Tube (provided). Expect around 10 ml of supernatant from 5 g of soil.
    OPTIONAL: For extracts that still contain suspended soil particulates, repeat Steps 10 and 11.

12. Add 0.25 ml of 100% TCA to each 1 ml of supernatant to precipitate protein.
13. Vortex or shake briefly to mix and incubate at –15 to –30°C for 1 h to overnight.
14. Thaw the 50 ml Falcon Collection Tube until the supernatant is liquid but still ice-cold.
15. Centrifuge the Falcon Collection Tube at 4500 x *g* in a refrigerated centrifuge (2–8°C) for 20 min. Pre-chill 1.7 ml Low-Protein Binding Tubes and a microcentrifuge to 2–8°C.
16. Remove as much liquid as possible without disturbing the pellet. If the pellet becomes dislodged, pipette off as much liquid as possible before proceeding. Discard the liquid.
17. Add 1 ml of ice-cold HPLC-grade acetone and completely resuspend the pellet by repeatedly pipetting and vortexing.
    Note: Keep samples on ice as much as possible and keep the acetone cold.
18. Transfer the acetone-suspended protein to a 1.7 ml Low-Protein Binding Tube (provided).
    Note: If a refrigerated centrifuge for 2 ml tubes is not available, the sample can be transferred to another 50 ml Falcon Collection Tube (user provided).
19. Centrifuge at 20,000 x *g* for 5 min in a refrigerated centrifuge (2–8°C). If a 50 ml Collection Tube was used in Step 18, centrifuge at 4500 x *g* for 10 min at 2–8°C.
20. Pour off the acetone while being careful to not dislodge the pellet. If the pellet becomes dislodged, remove acetone using a pipette tip.
21. Wash the pellet with 1 ml of ice-cold acetone and vortex for 10 s to resuspend.
22. Centrifuge at 20,000 x *g* for 5 min in a refrigerated centrifuge (2–8°C).
23. Repeat Steps 20–22. Then carefully pipet off the acetone, and dry the pellet in a hood or with N_2 gas until it is free of liquid but not crystallized.
    Note: Watch samples carefully. Pellets from soils high in organic material and darker pellets will take longer to dry (Drying time could vary from 5 to 60 min). When a pellet is dry, it will pull away from the side of the tube. If a pellet becomes too dry, it will be difficult to resuspend and may also be lost from the tube.
    Note: Keep dried pellets frozen (–15 to –30°C) until ready to proceed to next analysis.
24. Resuspend pellet in 25–200 µl of buffer of choice for downstream evaluation (e.g., Laemmli or Tris-HCl buffer for 1D gel visualization; ammonia bicarbonate, guanidine or urea buffer for 2D PAGE; trypsin digest prior to mass spectrophotometry).
25. Rigorous pipetting of the pellet is required to dissolve proteins. Please see the Appendix section of the Troubleshooting Guide for specific buffer formulations.
26. Your sample is now ready for 1D, 2D PAGE or 2D LC-MS/MS. Please see the Troubleshooting Guide if sample will be used for 1D LC-MS/MS.