MoBio PowerSoil-htp 96-Well Manual Extraction Method Using a Swing Bucket Centrifuge

Argonne National Laboratory

INTRODUCTION

This protocol describes how to extract DNA using MoBio 96-well PowerSoil DNA Extraction kits.
MOBIO Catalog # 12955-4 and 12955-12

MATERIALS PROVIDED IN THE POWERSOIL DNA EXTRACTION KIT (PER EXTRACTION)

(1) Bead plate
(1) Spin plate (filter)
(1) 0.5ml collection
(4) 1.0ml collection plates
(2) 2.0ml collection plates
(1) Microplate (DNA elution)
Sealing Tape
Centrifuge Tape
Elution Sealing Mat
Labeled solutions

MATERIALS NOT PROVIDED IN THE POWERSOIL DNA EXTRACTION KIT

Picogreen information
RNAaway Wipes
Molecular Biology Grade Ethanol
DNA decontaminating solution (DNAaway, 10% bleach, etc.)
Water bath (up to 65°C)
Ice bath
Disposable Polypropylene Weighing Funnels (VWR, Cat # DPWF-PP1-S)
Tweezers
Plastic sheets (See 'Loading the PowerSoil 96-Well Extraction Plate With Soil' Protocol)
Disposable Miltifunction Laboratory Spatulas (VWR, Cat #17211B)
Swing bucket centrifuge capable of 2500 x g
Plate shaker with 4 metal plate adapters, 2 black rubber bottom mats. This protocol uses the 96 well plate shaker (catalog #11996) listed on the MoBio website.
Multichannel Pipettes with a volume of up to 1200uL and corresponding tips
Sterile Liquid reservoirs
MicroAmp Clear Adhesive Film

LOADING THE POWERSOIL 96-WELL EXTRACTION PLATE WITH SOIL

1. Centrifuge Bead Plate for 1 min at 2500 x g to pellet the beads.

2. Remove the Square Well Mat from the PowerSoil®-htp Bead Plate and store in a temporary sterile location.

3. Because the plate will be out at room temperature for a long period of time during loading, it is advised to keep the bead plate on ice at all times. We place the bead plate on a solid rectangular chunk of dry ice in an ice bucket.

4. As to only expose one well at a time and to prevent soil from falling into inadvertent wells, it is recommended to use a sterilized cover for the plate. We modified plastic photo book sheets to fit the size

of the plate. In total we generated 96 of these sheets – one for each well on the bead plate. On each sheet, we used a hole puncher to punch out a hole that matches a particular well on the sample plate.

5. Each sheet is rinsed with DI water and left to dry. Before use, each sheet is rinsed with ethanol. The sheet is allowed to dry and then it is placed on top of the plate, with the puncture directly above the well that the plastic sheet corresponds to.

7. Using a Disposable Spatula, weigh out 0.15 to 0.25 grams of soil and put it through the Disposable Funnel and into the well.

8. Write down the exact weight of soil subsampled and the weight of residual soil left in funnel on a data sheet, in addition to sample ID, extraction date, technician initials and well ID.

9. Repeat this process for each well you wish to fill.

10. Once you are finished with loading the wells, refit the square well mat back to the bead plate and store at -80 degrees Celsius until you are ready to continue to the extraction protocol.

EXTRACTING DNA FROM PRE-LOADED POWERSOIL DNA EXTRACTION PLATE

Set water bath to 65ºC before starting protocol

1. Remove the pre-loaded (see above instruction for soil loading) PowerSoil®-htp Bead Plate from the freezer.

2. BEFORE THE FIRST USE ONLY, Solution C5-D must be prepared. Add an equal amount of 100% Ethanol to Solution C5-D (for the 4 prep kit = 120 ml, or for the 12 prep kit = 360 ml). Mix well. Put a check mark in the “ethanol added” box on the bottle cap label.

3. Add 750 µl of PowerSoil®-htp Bead Solution to the wells of the PowerSoil®-htp Bead Plate.

4. Check Solution C1. If Solution C1 has precipitated, heat solution at 60°C until the precipitate has dissolved.

> **Note:** Solution C1 contains SDS. If it gets cold, it will precipitate. Heating at 60°C will dissolve the SDS. Solution C1 can be used while it is still warm.

5. Add 60 µl of Solution C1. Secure the Square Well Mat (from step 2) tightly to the plate. Mix solution by inverting and shaking several times. Place sealed plates in 65°C water bath for 20 min. DO NOT SUBMERGE THE PLATES.

6. Place PowerSoil®-htp Bead Plate in Aluminum plates with 2-3 folder paper towels in between the Square Well Mat and the corresponding aluminum plate. We do this to securely fasten the PowerSoil®-htp Bead Plate to the 96 Well Plate Shaker to prevent spillage.

> **Note:** The final order of assembly is; Aluminum Plate, folded paper towels, 96 Well PowerSoil®-htp Plate, and second Aluminum Plate.

7. Shake at speed 20 for 10 minutes, rotate plates 180 degrees, and shake at speed 20 for another 10 minutes.

8. Centrifuge at room temperature for 30 minutes at 2500 x g.

a. While centrifuging, aliquot 250 µl of Solution C2 into each well of Plate #1 (1mL collection plate) and cover with Sealing Tape.

9. Remove the PowerSoil®-htp Bead Plate from the 96 Well Plate Shaker.

10. Carefully remove the Sealing Tape from Plate #1 and transfer the supernatant (~ 400-500µl) from the Bead Plate to Plate #1.

Note: The supernatant may still contain some soil particles.

11. Apply MicroAmp Clear Adhesive Film to Plate #1. This film is stickier than the sealing tape; therefore it should better prevent liquid from moving between wells when plate is shaken. Mix solution by inverting and shaking Plate #1 several times. Pressure should be applied to the adhesive film against Plate #1 to ensure there is no spillage. Vortex for 5 seconds (again applying pressure against the adhesive film to avoid spillage) and incubate at 4°C for 10 minutes in an ice bath. Centrifuge Plate #1 at room temperature for 10 minutes at 2500 x g.

12. After centrifugation, carefully remove and discard Adhesive Film from Plate #1.

13. Avoiding the pellet, transfer the entire volume (~600 µl depending on sample type) of supernatant in Plate #1 to Plate #2 (a fresh 1 mL collection plate). Always use 300 µl tips when transferring out of the 1 mL collection plates (the larger 1200 µL tips can cause overflow between wells). For each well, transfer two volumes of 300 µl into Plate #2.

14. Apply Sealing Tape to Plate #2 and centrifuge at room temperature for 10 minutes at 2500 x g. Since this plate is not being shaken it is okay to use the Sealing Tape instead of the stickier Adhesive Film.

a. While centrifuging, aliquot 200 µl Solution C3 into each well of Plate #3 (a fresh 1 mL collection plate), then cover with Sealing Tape.

15. Carefully remove Sealing Tape from Plate #2 and Plate #3. Avoiding the pellet, transfer the entire volume of supernatant (~600 µl) from Plate #2 to Plate #3 by aliquoting two volumes of 300µl into each fresh well.

16.Apply Adhesive tape to Plate #3. Vortex for 5 seconds and incubate at 4°C for 10 minutes in an ice bath. Centrifuge at room temperature for 10 minutes at 2500 x g.

17. Carefully remove and discard Adhesive Tape from Plate #3. Avoiding the pellet, transfer the entire volume of supernatant (~600 µl) to Plate #4 (a fresh 1 mL collection plate) by aliquoting two volumes of 300 µl into each fresh well.

18. Apply Sealing Tape to Plate #4 and centrifuge at room temperature for 10 minutes at 2500 x g.

a. While centrifuging, add 650 µl of Solution C4 to Plate #5 (a 2 mL collection plate).

19. Avoiding any residual pellet, transfer up to 650 µl of supernatant in Plate #4 (1 mL collection plate) to Plate #5 (2 mL collection plate).

20. Add a second 650 µl (1300 µl C4 total) aliquot of Solution C4 to each well of Plate #5.

Note: It is safe to stop the protocol at this step and store the samples covered with Sealing Tape at 4°C. Make sure to briefly centrifuge the plate to collect any condensate on the plate seal after overnight storage.

21. Pipet samples “up and down” to mix.

22. Place Spin Plate onto Plate #6.

23. Load approximately 650 µl from Plate #5 into each well of the Spin Plate and apply Centrifuge Tape.

24. Centrifuge at room temperature for 5 minutes at 2500 x g. Discard the flow through and place the Spin Plate back on Plate #6. Carefully remove and discard the Centrifuge Tape.

25. Repeat steps 23-24 until all the supernatant has been processed. Discard the final flow through.

26. Place the Spin Plate back on Plate #6.

27. **Confirm that ethanol has been added to Solution C5-D (see step 1)**. Add 500 µl of Solution C5-D to each well of the Spin Plate. Apply Centrifuge Tape to the Spin Plate.

28. Centrifuge at room temperature for 5 minutes at 2500 x g. Discard the flow through and place the Spin Plate back on Plate #6.

29. Centrifuge again at room temperature for 10 minutes at 2500 x g. Discard the flow through.

30. Carefully place the Spin Plate onto the Microplate. Remove Centrifuge Tape from the Spin Plate and discard.

31. Add 100 µl of Solution C6 (elution buffer) to the center of each well of the Spin Plate. To prevent elution buffer from getting caught on the side of wells, make sure to discharge liquid as close to filter tips as possible. Apply Centrifuge Tape. Let C6 sit on the filter for 15 minutes at room temperature before final centrifugation step.

32. Centrifuge at room temperature for 7 minutes at 2500 x g. Remove Centrifuge Tape and discard.

33. Aliquot 10 µl of DNA into a separate PCR plate to be used for PicoGreen quantification and PCR.

34. Cover wells of Microplate with the Elution Sealing Mat provided. DNA is now ready for any downstream application. No further steps are required.

Prolonged storage at 4°C will result in the evaporation of eluted DNA. We recommend storing DNA frozen (-20°C or -80°C). Solution C6 does not contain EDTA. To concentrate the DNA see the Hints and Troubleshooting Guide.