**PowerSoil 2x96**

MEMORANDUM FOR MicroLab, Berry Center 320/115

SUBJECT: Standard Operating Procedure for Assist Plus PowerSoil Tube Kits

REFERENCES:

1. Quick-Start Protocol: DNeasy PowerSoil Kit
2. SOP for Assist Plus

1. Purpose: To provide a standard for personnel practices within the Ecology and Biodiversity Laboratory Department. Updates to SOP will be provided as needed by authorized laboratory personnel, as instructed by supervisor (see signature block for supervisor).

2. Scope: This SOP of the Ecology and Biodiversity Laboratory Department Supervisor and Authorized Designees matches the instructions as seen in the **Quick-Start Protocol of the DNeasy PowerSoil Kit** to the instructions as seen on the **Assist Plus machine**. All personnel who are running samples with the PowerSoil Kits on the Assist Plus machine should be familiar with this SOP and all related references.

**The Bold Black Ink are the instructions to be followed when running a DNeasy Power Soil (50) or (100) on the AssistPlus robot.** All other text is for reference only.

**Note: Only Load Partial Tip Boxes as FIRST Box!! It is your only chance to tell the robot that a box is not full. Empty columns must be furthest left.**

3. Procedures and Actions:

**I. Assist Plus Machine Setup:**

1. **Open pipette tray, set on stand below arm with lid facing out**
   * **Slot A1 of pipette tray goes in back left corner of stand below arm**
2. **Select correct size reservoir (10 mL to start) and set on first stand**
   * **Ensure reservoir is parallel to pipette arm and seated firmly**
   * **Ensure reservoir of all sizes are available (10, 25, and 100 mL)**
3. **Set sample 1.5-2 mL tube racks on middle and right stands**

II. **Sample Preparation:**

1. Notes Before Starting
   * **Perform all centrifugation steps at room temperature (15-25°C)**
   * **If Solution C1 has precipitated, heat at 60°C until precipitate dissolves**
   * **Required Plates are found in kit**
   * **Label All Plates and MB Spin Column (Filter) with the same identifier as initial sample, one set for each sample.**
   * **One “kit blank” without any sample added should be run for each kit lot number.** 
     + **Record Lot Number Used**
   * **Save All Files in the proper folder found in the directory:**
     + Windows: [\\petalibrary.arcc.uwyo.edu\commons\EPSCoR\_Micro\MicroLab](file://petalibrary.arcc.uwyo.edu/commons/EPSCoR_Micro/MicroLab)
     + Mac: <smb://petalibrary.arcc.uwyo.edu/commons/EPSCoR_Micro/MicroLab>
2. Integra Assist Plus Protocol (List numbers below correspond to step #s on pipette)
   * **Select “Assist Plus”, then “ViaLab”, then “PowerSoil 2x96”**
   * **Samples are first loaded in both sample slots**
   1. Initial Volumes (Sets volumes of labware for tracking)
   2. **Load 100 ml Reservoir with 75 ml PowerBead**
   3. **Load Sample Plates in both slots (after spinning down!!!!!!)**
   4. Repeat Dispense 750ul to left hand plate (Powersoil Step 1)
   5. **Refill same 100 ml reservoir with 75 ml PowerBead**
   6. Volume Change for robot’s tracking purposes
   7. Repeat Dispense 750ul to right hand plate (Powersoil Step 1)
   8. **Load 10 ml Reservoir w 12 ml C1**
   9. Repeat Dispense 60ul to left hand plate (Powersoil Step 3)
   10. Repeat Dispense 60ul to right hand plate (Powersoil Step 3)
   11. **Add lids to plates, place in Mixer Mill Adaptors with lids on flat side** (Powersoil Step 4)
   12. **Shake Plates 20 Hz for 10 minutes, remove and swap orientation, then repeat** (Powersoil Step 5)
   13. **Centrifuge 6 minutes 4500 rcf** (Powersoil Step 6)
   14. **While Spinning, place new plates in both Slots and 50 ml C2 to new 100 ml Reservoir**
   15. Volume Change for robot’s tracking purposes
   16. Repeat Dispense 60ul to left hand plate (Powersoil Step 8)
   17. Repeat Dispense 60ul to right hand plate **then remove C2 resrvoir** (Powersoil Step 8)
   18. **Remove lefthand C2 plate. Replace with Sample Plate matching righthand C2’s label**
   19. Transfer supernatant from lefthand plate to righthand
   20. **Swap both plates for next set (sample left, C2 right)**
   21. Transfer supernatant from lefthand plate to righthand
   22. **Seal and Vortex plates for 5 seconds. Incubate 4C for 10 min** (Powersoil Step 9)
   23. **Centrifuge 6 minutes at 4500 rcf** (Powersoil Step 10)
   24. **Load new 1 ml plate to right hand side and Spun Plate 1 to lefthand**
   25. Volume Change for robot’s tracking purposes
   26. Transfer supernatant from lefthand plate to righthand (Powersoil Step 11)
   27. **Load new 1 ml plate to right hand side and Spun Plate 2 to lefthand**
   28. Transfer supernatant from lefthand plate to righthand (Powersoil Step 11)
   29. **Seal Plates. Centrifuge 6 minutes at 4500 rcf**
   30. **While Spinning, New Plates in both Spots. Load 40 ml C3 to 100 ml Reservoir**
   31. Volume Change for robot’s tracking purposes
   32. Repeat Dispense 200ul to left hand plate (Powersoil Step 13)
   33. Repeat Dispense 200ul to right hand plate (Powersoil Step 13)
   34. **Remove lefthand C3 plate. Replace with Sample Plate matching righthand C3’s label**
   35. Volume Change for robot’s tracking purposes
   36. Transfer supernatant from lefthand plate to righthand (Powersoil Step 12)
   37. **Swap both plates for next set (sample left, C3 right)**
   38. Transfer supernatant from lefthand plate to righthand (Powersoil Step 12)
   39. **Seal and Vortex plates for 5 seconds. Incubate 4C for 10 min**
   40. **Centrifuge 6 minutes at 4500 rcf**
   41. **Load new 1 ml plate to right hand side and Spun Plate 1 to lefthand**
   42. Volume Change for robot’s tracking purposes
   43. Transfer supernatant from lefthand plate to righthand (Powersoil Step 12)
   44. **Load new 1 ml plate to right hand side and Spun Plate 2 to lefthand**
   45. Transfer supernatant from lefthand plate to righthand (Powersoil Step 12)
   46. **Centrifuge 6 minutes at 4500 rcf**
   47. **Load New 2 ml plates to both slots. Add 65 ml C4 to 100 ml Reservoir**
   48. Volume Change for robot’s tracking purposes
   49. Repeat Dispense 650ul to left hand plate (Powersoil Step 15)
   50. **Refill C4 to 65 ml**
   51. Volume Change for robot’s tracking purposes
   52. Repeat Dispense 650ul to right hand plate (Powersoil Step 15)—have to add 65 mL here
   53. Volume Change for robot’s tracking purposes
   54. Repeat Dispense 650ul to lefthand plate(Powersoil Step 15)
   55. **Refill C4 to 65ml**
   56. Volume Change for robot’s tracking purposes
   57. Repeat Dispense 650 ul to right hand plate (Powersoil Step 15)
   58. **Remove righthand C4 plate. Replace with Sample Plate matching lefthand C4’s label**
   59. Transfer supernatant from righthand plate to lefthand (Powersoil Step 14)
   60. **Swap both plates for next set (sample right, C4 left)**
   61. Volume Change for robot’s tracking purposes
   62. Transfer supernatant from righthand plate to lefthand (Powersoil Step 14)
   63. **Remove right hand plate. Replace with filter matching lefthand label**
   64. Volume Change for robot’s tracking purposes
   65. Transfer supernatant from lefthand plate to righthand (Powersoil Step 17)
   66. **Swap both plates for next set (sample left, filter right)**
   67. Transfer supernatant from lefthand plate to righthand (Powersoil Step 17)
   68. **Centrifuge for 3 minutes at 4500xg. Keep all plates** (Powersoil Step 18)
   69. **Discard flow through. Return Matching sample 1 and filter 1**
   70. Volume Change for robot’s tracking purposes
   71. Transfer supernatant from lefthand plate to righthand (Powersoil Step 19)
   72. **Swap both plates for next set (sample left, filter right)**
   73. Transfer supernatant from lefthand plate to righthand (Powersoil Step 19)
   74. **Centrifuge for 3 minutes at 4500xg. Keep all plates**
   75. **Discard flow through. Return Matching sample 1 and filter 1**
   76. Volume Change for robot’s tracking purposes
   77. Transfer supernatant from lefthand plate to righthand (Powersoil Step 19)
   78. **Swap both plates for next set (sample left, filter right)**
   79. Transfer supernatant from lefthand plate to righthand (Powersoil Step 19)
   80. **Centrifuge for 3 minutes at 4500xg. Keep all plates**(Powersoil Step 19)
   81. **Discard flow through.**
   82. Volume Change for robot’s tracking purposes
   83. **Load 100 ml C5-D into 100ml reservoir. Check to make sure EtoH has been added.**
   84. **Return Plate 1 to righthand slot.**
   85. Transfer 500ul of C5 into righthand plate (Powersoil Step 21)
   86. **Return Plate 2 to righthand slot.**
   87. Transfer 500ul of C5 into righthand plate (Powersoil Step 21)
   88. **Seal with porous tape. Centrifuge for 3 minutes at 4500rcf** (Powersoil Step 22)
   89. **Discard flow through.**
   90. **Centrifuge for 5 minutes at 4500 rcf.** (Powersoil Step 23)
   91. **Discard flow through.**
   92. **Place filters on labeled elution plates.** (Powersoil Step 24)
   93. **Air dry for 10 minutes** (Powersoil Step 25)
   94. **Load 22ml of C6 into 25ml reservoir**
   95. Volume Change for robot’s tracking purposes
   96. **Load Plate 1 on rightside.**
   97. Transfer 100ul of C6 into righthand plate (Powersoil Step 26)
   98. **Replace Plate 1 with Plate 2 on righthand side.**
   99. Transfer 100ul of C6 into righthand plate (Powersoil Step 26)
   100. **Centrifuge for 3 minutes at 4500 xg** (Powersoil Step 27)
   101. **Save Flow Through. This is DNA**

IV. Troubleshooting, Care and Maintenance, Safety Precautions

**If Assist Plus prompts “Tips Still Detected” when tips are absent, wipe off both sensor sides with 95% EtOH**

1. Refer to SOP for Assist Plus Reservoir for all troubleshooting, care/maintenance, and safety precautions as needed.
2. Safety Data Sheets: [www.qiagen.com/safety](http://www.qiagen.com/safety).
3. Technical Assistance: support.qiagen.com.
4. The DNeasy PowerSoil Kit can be stored at room temperature (15-25°C) until the expiry date provided on the box label.

4. Signature Block

The following contact is the current assigned supervisor for the Ecology and Biodiversity Laboratory Department. Any questions regarding the information contained within this SOP or any problems found with the Assist Plus machine or any associated components should be directed to the following:

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