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 details 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. It is mostly taken from the manual instructions of the kit itself.

**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
   1. **Perform all centrifugation steps at room temperature (15-25°C)**
   2. **If Solution C1 has precipitated, heat at 60°C until precipitate dissolves**
   3. **2 mL collection tubes are found in kit**
   4. **Gather 48 or fewer randomized samples from each site for the study**
      * **The researcher should provide them loaded with soil sample**
      * **Scan barcodes into tracking document**
      * **Sort by Column (A1, B1, C1, D1, E1, F1, G1, H1, A2, B2 . . .)**
   5. **Label 4 Collection Tubes and 1 MB Spin Column (Filter) with the same identifier as initial sample, one set for each sample.**
   6. **One “kit blank” without any sample added should be run for each kit lot number.** 
      * **Record Lot Number Used**
   7. **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>
   8. **Always return tubes in the same order they were removed!!**
2. Quick-Start Protocol Instructions
   1. Add 0.25 g of soil sample to the PowerBead Tube provided. Gently vortex to mix. **(Samples should arrive with this already done)**
   2. Add 60 µL of Solution C1 and invert several times or vortex briefly to mix.
      * **Assist Plus Step #1: Load new reservoir (Solution C1)**
        + **Use 10mL reservoir: add [(60µL Solution x 50 samples) + 2,200µL dead volume] for total of 5.2mL minimum to reservoir. Use whole bottle or the minimum. This is the only reagent to return to the bottle after adding to samples**
      * **Assist Plus Step #2: Repeat dispense from well 🡺 left tray**
   3. Secure PowerBead Tubes horizontally using a Vortex Adapter tube holder (cat. No., 13000-V1-24).
      * **Assist Plus Step #3: Vortex**
        + **Vortex at maximum speed for 15 minutes (use 24 place adaptor). Add first sample to “1” spot and follow arrow clockwise around**
   4. Centrifuge tubes at 10,000 xg for 30 secs.
      * **Assist Plus Step #4: Centrifuge**
        + **Add first sample to “1” and follow numbers clockwise around.**
        + **Centrifuge 10,000 rcf for 30 secs**
   5. Transfer the supernatant to a clean 2 mL collection tube
      * **Place clean 2 mL collection tubes in right tray**
      * **Assist Plus Step #5: Transfer left tray 🡺 right tray**
      * **Assist Plus Step #7: Volume change (automatic)**
   6. Add 250µL of Solution C2 and vortex for 5 secs. Incubate at 4°C for 5 mins.
      * **Assist Plus Step #6: Load new reservoir (Solution C2)**
        + **Use 25mL reservoir: add full bottle or [(250µL x 50 samples) + 3,300µL dead volume] for total of 15.8mL to reservoir**
      * **Assist Plus Step #8: Repeat dispense from well 🡺 right tray**
      * **Assist Plus Step #9: Vortex**
        + **Vortex each tube briefly with standard vortex cup**
      * **Assist Plus Step #10: Incubate at 4C for 5 minutes (refrigerator)**
   7. Centrifuge the tubes for 1 min. at 10,000 x *g*.
      * **Assist Plus Step #11: Centrifuge**
        + **Add first sample to “1” and follow numbers clockwise around.**
        + **Centrifuge 10,000 rcf for 1 minute**
   8. Avoiding the pellet, transfer up to 600 µL of supernatant to a clean 2 mL collection tube.
      * **Assist Plus Step #12: New left plate, sample right**
        + **Place clean 2mL tubes in left tray and return sample tray to right spot**
      * **Assist Plus Step #13: Volume change (automatic)**
      * **Assist Plus Step #14: Transfer from right tray 🡺 left tray**
   9. Add 200 µL of Solution C3 and vortex briefly. Incubate at 4°C for 5 mins.
      * **Assist Plus Step #15: Load new reservoir (Solution C3)** 
        + **Use 25mL reservoir: add [(200µL x 50 samples) + 3,300µL dead volume] for a minimum of 13.3mL to reservoir. Use full bottle if available**
      * **Assist Plus Step #16: Volume change (automatic)**
      * **Assist Plus Step #17: Repeat dispense from well 🡺 left tray**
      * **Assist Plus Step #18: Vortex**
        + **Vortex each tube briefly with standard vortex cup**
      * **Assist Plus Step #19: Incubate at 4C for 5 minutes (refrigerator)**
   10. Centrifuge the tubes for 1 min. at 10,000 x *g*.
       * **Assist Plus Step #20: Centrifuge**
         + **Add first sample to “1” and follow numbers clockwise around.**
         + **Centrifuge 10,000 rcf for 1 minute**
   11. Avoiding the pellet, transfer up to 750 µL of supernatant to a clean 2 mL collection tube.
       * **Assist Plus Step #21: Sample Right, New Plate Left**
         + **Place clean 2mL tubes in left tray, Load sample tray to right**
       * **Assist Plus Step #22: Volume change (automatic)**
       * **Assist Plus Step #23: Transfer from right tray 🡺 left tray**
   12. Shake to mix Solution C4 and add 1200 µL to the supernatant. Vortex for 5 secs.
       * **Assist Plus Step #24: Load new reservoir (Solution C4)**
         + **Use 100mL reservoir: add [(1200µL x 50 samples) + 3,000µL dead volume] for total of 63.0mL to reservoir**
       * **Assist Plus Step #25: Volume change (automatic)**
       * **Assist Plus Step #26: Repeat dispense from well 🡺 left tray**
       * **Assist Plus Step #27: Vortex**
         + **Vortex each tube briefly with standard vortex cup**
       * **Assist Plus Step #28: Centrifuge for 8 seconds**
         + **Add first sample to “1” and follow numbers clockwise around.**
         + **Press “short” until it counts to 8**
   13. Load 675µL onto an MB Spin Column and centrifuge at 10,000 x *g* for 1 min. Discard flow through.
       * **Assist Plus Step #29: Load filter and 2 ml tube Right Positi**
         + **Add MB Spin Columns to right tray**
         + **Return samples to left tray**
       * **Assist Plus Step #30: Volume change (automatic)**
       * **Assist Plus Step #31: Transfer from left tray 🡺 right tray**
       * **Assist Plus Step #32: Centrifuge 1 min 10,000 rcf**
         + **Add first sample to “1” and follow numbers clockwise around.**
         + **Centrifuge 10,000 rcf for 1 minute**
       * **Assist Plus Step #33: Discard flow through**
         + **Dump Liquid underneath filter into waste container**
         + **Return filter to same, now empty, tube**
   14. **Repeat Step 13 twice, until all of the sample has been processed.** 
       * **Assist Plus Step #34-40: Refer to Step 13 (above)**
       * **DO NOT DISCARD TUBES ON LEFT UNTIL EMPTY!! (THIRD DRAW)**
   15. Add 500 µL of Solution C5. Centrifuge for 30 secs. At 10,000 x *g*.
       * **Assist Plus Step #41: Load new reservoir (Solution C5)**
         + **Use 25mL reservoir: add full bottle or [(500µL x 50 samples) + 3,000µL dead volume] for a minimum of 28.0mL to reservoir,**
       * **Assist Plus Step #42: Volume change (automatic)**
       * **Assist Plus Step #43: Repeat dispense from well 🡺 right tray**
       * **Assist Plus Step #44: Centrifuge 30 seconds 10,000 rcf**
         + **Add first sample to “1” and follow numbers clockwise around.**
         + **Centrifuge 10,000 rcf for 1 minute**
   16. Discard the flow through. Centrifuge again for 1 min. at 10,000 x *g*.
       * **Assist Plus Step #45:** **Discard flow through**
         + **Dump Liquid underneath filter into waste container**
         + **Return filter to same, now empty, tube**
       * **Assist Plus Step #46: Centrifuge 1 minute 10,000 rcf**
         + **Add first sample to “1” and follow numbers clockwise around.**
         + **Centrifuge 10,000 rcf for 1 minute**
   17. Carefully place the MB Spin Column into a clean 2 mL collection tube. Avoid splashing and Solution C5 onto the column.
       * **Transfer MB Spin Column to last labeled clean 2ml tube and return tray to right position**
       * **Assist Plus Step #47: Load new reservoir (Solution C6)**
         + **Use 10mL reservoir: add [(100µL x 50 samples) + 2,200µL dead volume] for a minimum of 7.5mL to reservoir and max full bottle**
       * **Assist Plus Step #48: Volume change (automatic)**
   18. Add 100 µL of Solution C6 to the center of the white filter membrane. Alternatively, you can use sterile DNA-Free PCR Grade Water for this step (cat. No. 17000-10)
       * **Assist Plus Step #49: Repeat dispense from well 🡺 right tray**
   19. Centrifuge at room temperature for 30 secs. At 10,000 x g. Discard the MB Spin Column. The DNA is now ready for downstream applications.
       * **Assist Plus Step #50: Centrifuge 30 seconds 10,000 rcf**
         + **Add first sample to “1” and follow numbers clockwise around.**
         + **Centrifuge 10,000 rcf for 30 secs**

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: