PROTOCOL

FastDNA™ SPIN Kit for Soil

Cat. No. 116560200/116560300
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IMPORTANT ▸ *Before using SEWS-M wash solution, add 100 mL of 100% ethanol and mark on the bottle label the date ethanol was added.*

☐ **1** Add up to **500 mg** of soil sample to a Lysing Matrix E tube.

NOTE ▸ *See section 3 in the User Manual for other important guidelines*

☐ **2** Add **978 µL** Sodium Phosphate Buffer to sample in Lysing Matrix E tube.

☐ **3** Add **122 µL** MT Buffer to solubilize external contaminants.

☐ **4** Homogenize in the FastPrep instrument for **40 seconds** at a speed setting of **6.0 m/s** to disrupt cell wall and release nucleic acids.

☐ **5** Centrifuge at **14,000 x g** for **5–10 minutes** to pellet debris, such as insoluble cellular debris and lysing matrix.

NOTE ▸ *Extending centrifugation to 15 minutes can enhance elimination of excessive debris from large samples or from cells with complex cell walls.*

☐ **6** Transfer supernatant to a clean 2.0 mL microcentrifuge tube. Add **250 µL** PPS to separate the solubilized nucleic acids from the cellular debris and lysing matrix. Mix by inverting the tube 10 times.

☐ **7** Centrifuge at **14,000 x g** for **5 minutes** to pellet precipitate, removing the cellular debris and lysing matrix. Transfer supernatant to a clean 15 mL microcentrifuge tube.

NOTE ▸ *While a 2.0 mL microcentrifuge tube may be used at this step, more efficient mixing and DNA binding will occur in a larger tube.*

☐ **8** Resuspend the Binding Matrix suspension and add **1.0 mL** to the supernatant in the 15 mL tube.

- [ ] **9** Place on rotator or invert by hand for **2 minutes** to allow binding of DNA to the Binding Matrix. Place tube on a rack for **3 minutes** to allow settling of Binding Matrix.
- [ ] **10** Remove and discard **500 µL** of supernatant being careful to avoid settled Binding Matrix.
- [ ] **11** Gently resuspend Binding Matrix in the remaining amount of supernatant. Transfer approximately **600 µL** of the mixture to a SPIN filter and centrifuge at **14,000 x g** for **1 minute**. Empty the catch tube and reuse. Then repeat with the remaining mixture.
- [ ] **12** Add **500 µL** prepared SEWS-M (with the appropriate amount of ethanol added) to further solubilize impurities. Gently resuspend the pellet using the force of the liquid from the pipet tip.
- [ ] **13** Centrifuge at **14,000 x g** for **1 minute** to remove impurities. Empty the catch tube and reuse.
- [ ] **14** Without addition of any liquid, centrifuge a second time at **14,000 x g** for **2 minutes** to “dry” the Binding Matrix of residual wash solution. Replace the catch tube with a new, clean catch tube.
- [ ] **15** Air dry the SPIN Filter for **5 minutes** at room temperature.
- [ ] **16** Gently resuspend Binding Matrix (above the SPIN Filter) with **50–100 µL** of DES.

  NOTE *To avoid over-dilution of the purified DNA, use smallest amount of DES required to resuspend Binding Matrix pellet.*

  NOTE *Yields may be increased by incubation for 5 minutes at 55 °C in a heat block or water bath.*
- [ ] **17** Centrifuge at **14,000 x g** for **1 minute** to bring eluted DNA into the clean catch tube. Discard the SPIN Filter.

DNA is now ready for your analysis and downstream applications.
Store at -20 °C for extended periods or 4 °C until use.