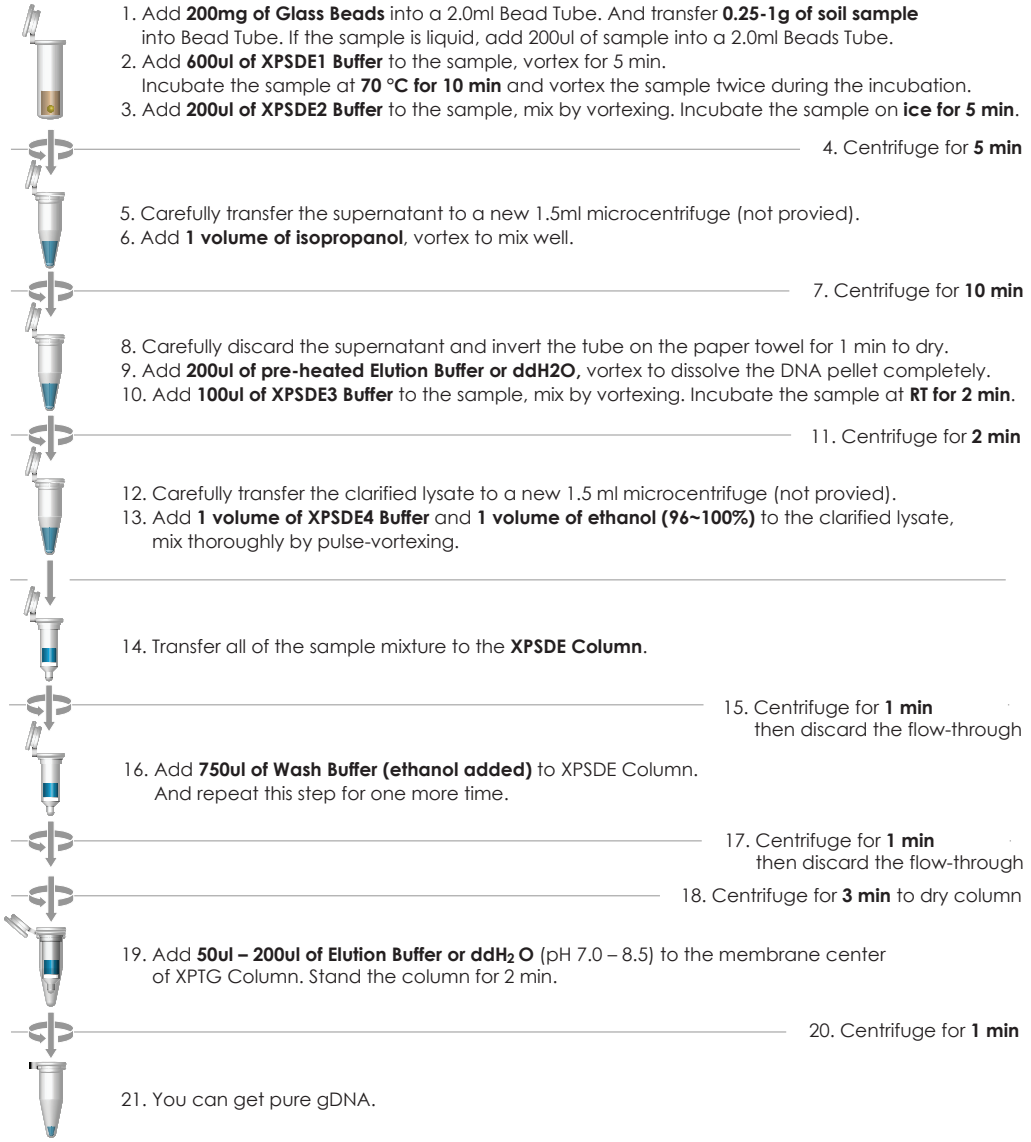
eCube Soil DNA Mini Kit

*** Things to do before starting**

1. Add 1.1 ml ddH_2O to each Proteinase K tube to make a 10mg/ml stock solution. Store the stock solution at 2-8°C.
2. For 50preps, add 80ml ethanol (96-100%) to Wash Buffer. For 200preps, add 320ml ethanol (96-100%) to Wash Buffer.
3. Prepare a water baths to 70 °C before the operation.
4. All centrifuge steps are done at full speed **(14,000 rpm or 10,000 x g)** in a microcentrifuge.



1. Add **200mg of Glass Beads** into a 2.0ml Bead Tube. And transfer **0.25-1g of soil sample** into Bead Tube. If the sample is liquid, add 200ul of sample into a 2.0ml Beads Tube.
2. Add **600ul of XPSDE1 Buffer** to the sample, vortex for 5 min. Incubate the sample at **70 °C for 10 min** and vortex the sample twice during the incubation.
3. Add **200ul of XPSDE2 Buffer** to the sample, mix by vortexing. Incubate the sample on **ice for 5 min**.
4. Centrifuge for **5 min**
5. Carefully transfer the supernatant to a new 1.5ml microcentrifuge (not provied).
6. Add **1 volume of isopropanol**, vortex to mix well.
7. Centrifuge for **10 min**
8. Carefully discard the supernatant and invert the tube on the paper towel for 1 min to dry.
9. Add **200ul of pre-heated Elution Buffer or ddH2O,** vortex to dissolve the DNA pellet completely.
10. Add **100ul of XPSDE3 Buffer** to the sample, mix by vortexing. Incubate the sample at **RT for 2 min**.
11. Centrifuge for **2 min**
12. Carefully transfer the clarified lysate to a new 1.5 ml microcentrifuge (not provied).
13. Add **1 volume of XPSDE4 Buffer** and **1 volume of ethanol (96~100%)** to the clarified lysate, mix thoroughly by pulse-vortexing.
14. Transfer all of the sample mixture to the **XPSDE Column**.
15. Centrifuge for **1 min** then discard the flow-through
16. Add **750ul of Wash Buffer (ethanol added)** to XPSDE Column. And repeat this step for one more time.
17. Centrifuge for **1 min** then discard the flow-through
18. Centrifuge for **3 min** to dry column
19. Add **50ul – 200ul of Elution Buffer or ddH_2O** (pH 7.0 – 8.5) to the membrane center of XPTG Column. Stand the column for 2 min.
20. Centrifuge for **1 min**
21. You can get pure gDNA.

Please consult the eCube Stool/Soil DNA Mini Kit Handbook before using these protocols for the first time.

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BENCH-TOP PROTOCOLS for eCube Soil DNA Mini Kit

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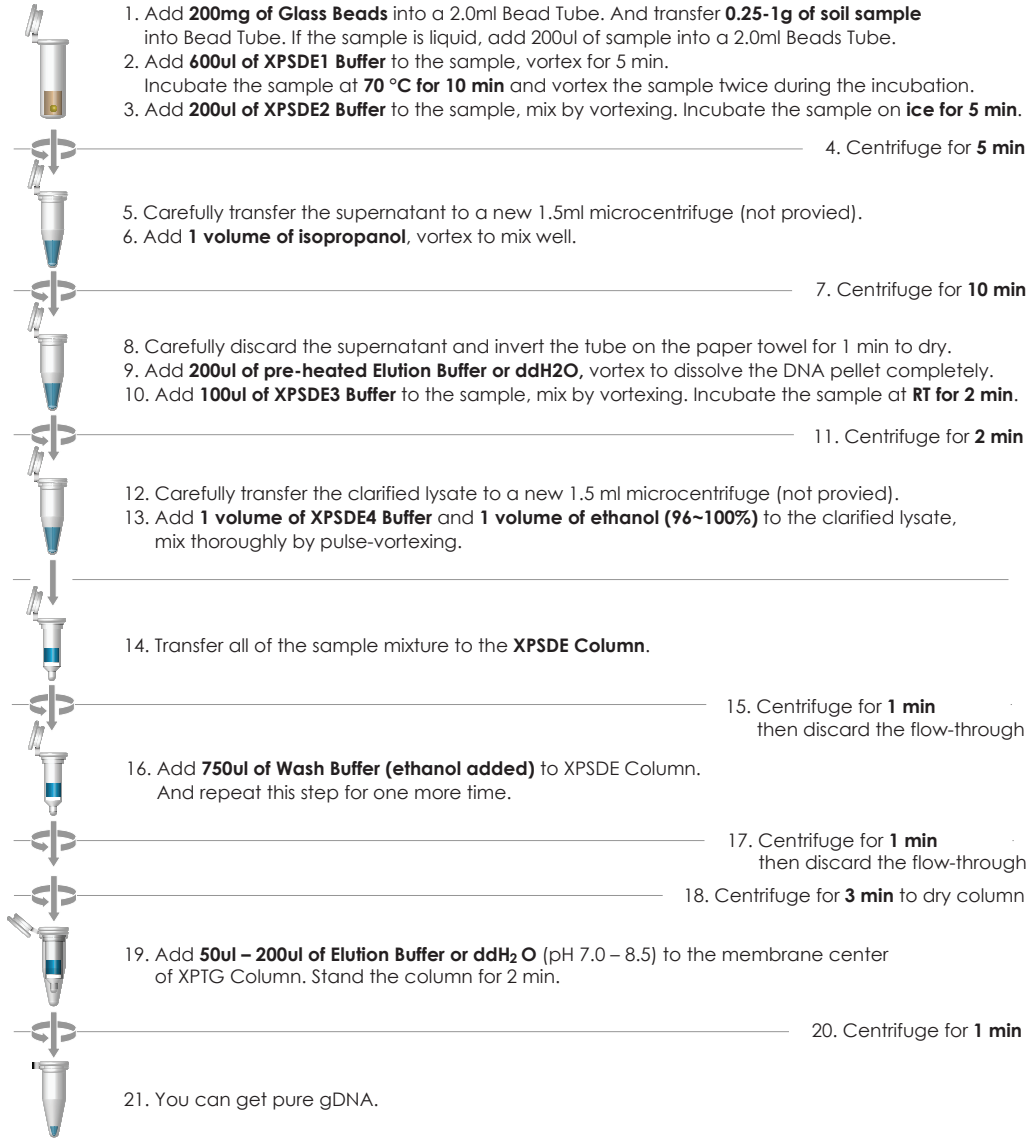

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7. Centrifuge for **10 min**
8. Carefully discard the supernatant and invert the tube on the paper towel for 1 min to dry.
9. Add **200ul of pre-heated Elution Buffer or ddH2O,** vortex to dissolve the DNA pellet completely.
10. Add **100ul of XPSDE3 Buffer** to the sample, mix by vortexing. Incubate the sample at **RT for 2 min**.
11. Centrifuge for **2 min**
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13. Add **1 volume of XPSDE4 Buffer** and **1 volume of ethanol (96~100%)** to the clarified lysate, mix thoroughly by pulse-vortexing.
14. Transfer all of the sample mixture to the **XPSDE Column**.
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