

# SUPPLEMENTARY PROTOCOL

Ver 1.0

**KIT-9060**

## **PrimeWay Soil DNA Extraction Kit**

A support protocol is an extra guide provided with the main protocol to help users use the product with special or less common sample types, workflows, or conditions. It helps expand the use of the main kit by giving tested methods for other applications, while still ensuring good performance and quality. It doesn't replace the main protocol but offers extra help for different situations.





## List of Supplementary Protocol for KIT-9060

1. Coral Samples



## 1. Coral Samples

### Materials Supplied by User

- ✓ 0.5 EDTA Solution, pH 8.0
- ✓ Water, Biotechnology Grade

Sample Pre-treatment	<ol style="list-style-type: none"> <li>I. Prepare a 50mM EDTA solution. For example, mix 4 mL of 0.5M EDTA with 36mL Milli-Q water to obtain 40mL of 50mM EDTA.</li> <li>II. Transfer samples (approximately 5 to 10mL) into a 50mL falcon tube.</li> <li>III. Add 40mL of 50mM EDTA.</li> <li>IV. Vortex at full speed for 15 minutes.</li> <li>V. [Optional] Centrifuge at 6000 rpm for 5 to 15 minutes.</li> </ol>
Sample	<ol style="list-style-type: none"> <li>1. [Optional] Pre-filter the water sample with bigger membrane filter pore size (e.g., 100 <math>\mu\text{m}</math>) to remove foreign materials and sediment.</li> <li>2. Perform vacuum filtration with the <b>300 – 500 mL water</b> sample, using 0.22 <math>\mu\text{m}</math> or 0.45 <math>\mu\text{m}</math> pore size, 25 mm membrane filter. If the membrane filter size is too big, excise the membrane filter to 25 mm diameter after filtration.</li> <li>3. Insert the membrane filter into the Soil Beads Tube by rolling the membrane, with the side containing trapped sample facing inward of the tube.  <b>Note:</b> <i>If sample is not process immediately after Step 3, the <b>Soil Bead Tube</b> with the membrane filter can be stored at -20 °C. To continue the DNA extraction, thaw the sample to room temperature before start.</i> </li> </ol>



Lysis	<ol style="list-style-type: none"><li>4. Add <b>800 <math>\mu\text{L}</math> SL1 Buffer</b> and <b>60 <math>\mu\text{L}</math> SL2 Buffer</b> into the <b>Soil Bead Tube</b>.</li><li>5. Disrupt the sample with max speed (2,850 rpm) for 10 minutes.</li><li>6. Short spin for 5 seconds to bring down the mixture and incubate at 65 °C (water bath) for 10 minutes.</li><li>7. Centrifuge at 13,000 x <i>g</i> for 1 minute.</li><li>8. Remove the membrane filter from the <b>Soil Bead Tube</b> with forceps.</li><li>9. Transfer <b>600 <math>\mu\text{L}</math> supernatant</b> into a new 1.5 mL microcentrifuge tube.</li><li>10. Add <b>150 <math>\mu\text{L}</math> SL3 Buffer</b>.</li><li>11. Vortex to mix and incubate for 3 minutes at room temperature.</li><li>12. Centrifuge at maximum speed (<math>\geq 13,000</math> x <i>g</i>) for 5 minutes.</li></ol>
Binding	<ol style="list-style-type: none"><li>13. Transfer the <b>clear supernatant (~750 <math>\mu\text{L}</math>)</b> to a new 2 mL microcentrifuge tube.</li><li>14. Add <b>equal volume of SBD Buffer (~750 <math>\mu\text{L}</math>)</b> and mix by inverting the tube 4 – 6 times.</li><li>15. Short spin to bring down the lysate.</li><li>16. Place a <b>PrimeWay Soil Column</b> into a new <b>Collection Tube</b>.</li><li>17. Transfer <b>up to 750 <math>\mu\text{L}</math> lysate</b> into the <b>PrimeWay Soil Column</b> and centrifuge at 10,000 x <i>g</i> for 1 minute. Discard the flow-through and place the column back into the <b>Collection Tube</b>.</li><li>18. Repeat Step 17 until all the sample has been transferred to the <b>PrimeWay Soil Column</b>.</li></ol>



Washing	<p>19. Add <b>500 <math>\mu</math>L Wash Buffer S1</b> to the <b>PrimeWay Soil Column</b> and incubate for 1 minute. Centrifuge at 10,000 x <i>g</i> for 1 minute. Discard the flow-through and place the column back into the <b>Collection Tube</b>.</p> <p>20. Add <b>650 <math>\mu</math>L Wash Buffer S2</b> to the <b>PrimeWay Soil Column</b>. Centrifuge at 10,000 x <i>g</i> for 1 minute. Discard the flow-through and place the column back into the <b>Collection Tube</b>.</p> <p>21. Repeat Step 20.</p>
Drying	<p>22. Centrifuge the column at 10,000 x <i>g</i> for 1 minute to dry the membrane.</p>
Elution	<p>23. Transfer the <b>PrimeWay Soil Column</b> to a new 1.5 mL microcentrifuge tube.</p> <p>24. Add <b>50 <math>\mu</math>L preheated Elution Buffer (65 °C)</b> to the center of the <b>PrimeWay Soil Column</b> membrane. Incubate at room temperature for 3 minutes. Centrifuge at 10,000 x <i>g</i> for 1 minute to elute the DNA.</p> <p>25. Store the eluted DNA at 2 – 8 °C or -20 °C for long-term storage.</p>