

SUPPLEMENTARY PROTOCOL

Ver 1.0

KIT-9070

PrimeWay Stool 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-9070

1. Stool stored in DNA/RNA Shield™



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Materials Supplied by User

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

Preparation	<ol style="list-style-type: none"> 1. Preheat the Elution Buffer at 60°C.
Sample	<ol style="list-style-type: none"> 1. Vortex the stool sample stored in DNA/RNA Shield™ vigorously for 30 seconds to ensure thorough homogenization. Using wide-bore pipette tips, transfer 200 µL of the stool slurry into the Stool Bead Tube. <p><i>Note: DO NOT transfer undigested food such as crop, fruit husks and undigested seeds into the Stool Bead Tube.</i></p>
Lysis	<ol style="list-style-type: none"> 2. Add 800 µL STL1 Buffer. 3. Vortex to mix and incubate at 70°C for 5 minutes. 4. Homogenise the sample with maximum speed using cell disruptor (e.g., Digital Disruptor Genie, 2850 rpm) for 20 minutes. 5. Centrifuge at 8,000 x g for 2 minutes. 6. Transfer 500 µL supernatant into a new 1.5 mL microcentrifuge tube (not provided). 7. Add 150 µL STL2 Buffer and vortex to mix for 5 seconds.



Lysis	<p>8. Incubate on ice (0 – 4°C) for 5 minutes.</p> <p>9. Centrifuge at 16,000 x g for 3 minutes.</p>
Inhibitor Removal	<p>10. Place an Inhibitor Removal Column (purple ring) into a 2 mL Microcentrifuge Tube.</p> <p>11. Transfer 500 µL clear supernatant into the Inhibitor Removal Column and centrifuge at 16,000 x g for 1 minute.</p> <p>12. KEEP THE FLOW-THROUGH and discard the column.</p> <p><i>Note: If pellet is observed in the flow-through, transfer the clear supernatant to a new 2 mL microcentrifuge tube (not provided).</i></p> <p>13. [Optional] Perform RNase treatment if RNA-free DNA is required. Add 4 µL RNase A (not provided) and incubate for 5 minutes at room temperature.</p>
Binding	<p>14. Add 800 µL STL3 Buffer to the flow-through and immediately shake vigorously for 5 seconds.</p> <p>15. Short spin to bring down the lysate.</p> <p>16. Place a PrimeWay Stool Column (green ring) into a new Collection Tube.</p> <p>17. Transfer up to 700 µL lysate into the PrimeWay Stool Column and centrifuge at 16,000 x g for 1 minute. Discard the flow-through and place the column back into the Collection Tube.</p> <p>18. Repeat Step 17 until all the lysate has been transferred to the PrimeWay Stool Column.</p>



Washing	<p>19. Add 400 μL STL3 Buffer to the PrimeWay Stool Column. Centrifuge at 16,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>20. Add 600 μL Wash Buffer ST to the PrimeWay Stool Column. Centrifuge at 16,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>21. Repeat Step 20.</p>
Drying	<p>22. Centrifuge the column at 16,000 x <i>g</i> for 3 minutes to dry the membrane.</p>
Elution	<p>23. Transfer the PrimeWay Stool Column to a new 1.5 mL microcentrifuge tube (not provided).</p> <p>24. Add 30 – 100 μL preheated Elution Buffer (60°C) to the centre of the PrimeWay Stool Column membrane. Incubate at room temperature for at least 2 minutes. Centrifuge at 16,000 x <i>g</i> for 2 minutes to elute the DNA.</p> <p>25. Store the eluted DNA at 2 – 8°C or –20°C for long-term storage.</p>

