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SUPPLEMENTARY PROTOCOL

Ver 1.0

KIT-9022

PrimeWay Genomic II 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-9022

1. Formalin-Fixed paraffin Embedded (FFPE) Tissue
2. Formalin-Fixed Tissue
3. Microbial bacteria DNA from saliva

1. Formalin-Fixed paraffin Embedded (FFPE) Tissue

Materials Supplied by User

- ✓ Undenatured absolute ethanol (≥ 99.5%)
- ✓ Nuclease-free water
- ✓ Vortex mixer
- ✓ Centrifuge, at speed of 16,000 – 18,000 x *g*
- ✓ Water bath or dry bath
- ✓ Pipettes & pipette tips
- ✓ 1.5 mL & 2 mL microcentrifuge tubes
- ✓ Xylene

Preparation	I. Set water bath/ dry bath to 37 °C, 60 °C and 90 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Transfer up to **25 mg FFPE Tissue** to a new 1.5 mL microcentrifuge tube. 2. Add **1.2 mL xylene** (not provided) and vortex vigorously. 3. Incubate at room temperature for 10 minutes. 4. Centrifuge at max speed (16,000 – 18,000 x *g*) for 5 minutes. Remove the supernatant. 5. Add **1.2 mL absolute ethanol** (not provided) and gently invert to mix. Centrifuge at max speed (16,000 – 18,000 x *g*) for 5 minutes.

Sample	6. Remove the supernatant. Repeat Step 5. 7. Remove supernatant completely. Incubate at 37 °C for 10 – 15 minutes, until the excess ethanol has evaporated.
Lysis	8. Add **200 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix. 9. Incubate at 60 °C for 1 hour. Invert the tube occasionally. 10. Incubate at 90 °C for 1 hour. 11. Centrifuge at 14,000 x *g* for 2 minutes to pellet insoluble debris. 12. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. 13. Add **200 µL GL2 Buffer**. Vortex to mix.
Binding	14. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 15. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 16. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 17. Discard the Collection Tube and place the column into a new Collection Tube.

Washing	18. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 19. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	20. Centrifuge the column at 14,000 x g for 3 minutes to dry the membrane.
Elution	21. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 22. Add **100 µL preheated Elution Buffer** to the center of the column membrane. Incubate at room temperature for at least 3 minutes. 23. Centrifuge at 14,000 x g for 30 seconds to elute the DNA.

2. Formalin-Fixed Tissue

Materials Supplied by User

- ✓ Undenatured absolute ethanol (≥ 99.5%)
- ✓ Nuclease-free water
- ✓ Vortex mixer
- ✓ Centrifuge, at speed of 14,000 x g
- ✓ Water bath or dry bath
- ✓ Pipettes & pipette tips
- ✓ 1.5 mL & 2 mL microcentrifuge tubes
- ✓ 1X Phosphate Buffered Saline (PBS)

Preparation	I. Set water bath/ dry bath to 60 °C and 90 °C. II. Preheat the **Elution Buffer** at 60 °C.
Sample	1. Wash the tissue with **1 mL PBS** with total of 2 times. Remove the liquid completely. 2. Mince the tissue into tiny pieces with sterile blade and transfer up to 25 mg tissue into a new 1.5 mL microcentrifuge.
Lysis	3. Add **200 µL GL1 Buffer** and **20 µL Proteinase K Solution**. Vortex to mix. 4. Incubate at 60 °C for 1 hour. Invert the tube occasionally. 5. Incubate at 90 °C for 1 hour.

Lysis	6. Centrifuge at 14,000 x *g* for 2 minutes to pellet insoluble debris. 7. Transfer the supernatant to a new 1.5 mL microcentrifuge tube. 8. Add **200 µL GL2 Buffer**. Vortex to mix.
Binding	9. Add **200 µL absolute ethanol** (not provided). Vortex to mix immediately. 10. Place a **PrimeWay Genomic II Column** into a new Collection Tube. 11. Transfer **up to 750 µL lysate**, including the precipitate if any, to the **PrimeWay Genomic II Column**. Centrifuge at 14,000 x *g* for 1 minute. 12. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	13. Add **400 µL Wash Buffer G1** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 14. Add **600 µL Wash Buffer G2** to the column. Centrifuge at 14,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	15. Centrifuge the column at 14,000 x g for 3 minutes to dry the membrane.

Elution	
	16. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 17. Add **100 µL preheated Elution Buffer** to the center of the column membrane. Incubate at room temperature for at least 3 minutes. 18. Centrifuge at 14,000 x g for 30 seconds to elute the DNA.

3. Microbial bacteria DNA from saliva sample

Materials Supplied by User

- ✓ 1x PBS
- ✓ Bacteria Pre-Lysis Buffer [20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0]
- ✓ Lyophilised lysozyme
- ✓ 50 mM EDTA, pH 8.0

Preparation	I. Set water bath/ dry bath to 60 °C. II. Preheat the Elution Buffer at 60 °C. III. **Bacteria Pre-Lysis Buffer Mix preparation:** Dissolve **20 mg Lysozyme** (not provided) into **1 mL Bacteria Pre-Lysis Buffer** (not provided).
Sample	1. Collect at least 1 mL saliva in a new 50 mL centrifuge tube. 2. Transfer **1 mL saliva** to a new 15 mL centrifuge tube. Add **5 mL PBS** (not provided). Vortex to mix vigorously. 3. Centrifuge at 2,000 x *g* for 5 minutes at room temperature to pellet cells. Immediately, discard the supernatant. 4. Weigh the saliva pellet to ensure it is approximately **20–40 mg.**

Sample	***Note**: If the pellet exceeds 40 mg, double the volume of all subsequent buffers up to the ethanol addition step. Proceed by loading the entire sample onto a single spin column.*
Lysis	5. Resuspend the pellet with **420 µL 50 mM EDTA** (not provided). Transfer the sample to a new 1.5 mL microcentrifuge tube. 6. Add **180 µL freshly prepared Bacteria Pre-Lysis Buffer Mix** (not provided, refer to preparation method section). Incubate at 37 °C for 30 – 60 minutes. 7. Centrifuge at 16,000 x g for 2 minutes. Discard supernatant. 8. Resuspend the pellet with **200 µL GL1 Buffer.** 9. Add **20 µL Proteinase K Solution**. Vortex to mix. 10. Incubate at 60 °C for 60 minutes. Invert the tube occasionally. 11. Add **200 µL GL2 Buffer**. Vortex to mix. 12. Add **4 µL RNase A Solution**. Vortex to mix 13. Incubate at room temperature for 5 minutes.
Binding	14. Add 200 µL of absolute ethanol (not provided). Vortex to mix immediately. 15. Place a PrimeWay Genomic II Column into a new Collection Tube. 16. Transfer up to 750 µL lysate, including the precipitate if any, to the PrimeWay Genomic II Column. Centrifuge at 14,000 x g for 1 minute.

	17. Discard the Collection Tube and place the column into a new Collection Tube.
Washing	18. Add 400 µL Wash Buffer G1 to the column. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the column back into the Collection Tube. 19. Add 600 µL Wash Buffer G2 to the column. Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.
Drying	20. Centrifuge the column at 14,000 x g for 3 minutes to dry the membrane.
Elution	21. Transfer the **PrimeWay Genomic II Column** to a new 1.5 mL microcentrifuge tube. 22. Add 5**0 µL preheated Elution Buffer** to the centre of the column membrane. Incubate at room temperature for at least 3 minutes. 23. Centrifuge at 14,000 x g for 30 seconds to elute the DNA.

Product Ordering Information

Protocol	Part Number	Product Description	Remarks
FFPE tissues	1st BASE BUF-2041-1x500ml	1X Phosphate Buffered Saline (PBS), Biotechnology Grade, 500 mL	