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SUPPLEMENTARY PROTOCOL

Ver 1.0

KIT-9080

PrimeWay Food 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-9080

1. Concentrated Fruit Juice
2. Oil

1. Concentrated Fruit Juice

Materials Supplied by Users

- ✓ Chloroform
- ✓ Vortex mixer
- ✓ Centrifuge, at speed of 8,000 – 16,000 x *g*
- ✓ Water bath or thermoblock
- ✓ Thermomixer (DLAB HM100-Pro LCD digital Thermo Mix, or similar)
- ✓ Ice bath
- ✓ 1 mL syringe
- ✓ 1.5 mL microcentrifuge tube
- ✓ 2 mL microcentrifuge tube
- ✓ 2 mL screw cap tube
- ✓ Pipettes & pipette tips

Protocol

Preparation

✓ Preheat the **Elution Buffer** at 65 °C.

Sample	1. Using a syringe (not provided), transfer **200 – 500 mg juice sample** into a new 2 mL microcentrifuge tube (not provided).
Lysis	2. Add **900 µL F1 Buffer** and **20 µL Proteinase K Solution**. Vortex vigorously to mix for 30 seconds or until homogenised. 3. Incubate the sample in thermomixer at 65 °C with constant shaking (1,000 rpm) for 60 minutes. 4. Add **300 µL F2 Buffer**. Vortex to mix for 30 seconds or until homogenised.

Lysis	5. Incubate the sample on ice (0 – 4 °C) for 5 minutes. 6. Centrifuge at 13,000 x *g* for 5 minutes. 7. Carefully transfer **700 µL clear supernatant** to a new 2 mL microcentrifuge tube (not provided). 8. Add **500 µL chloroform** (not provided). Vortex vigorously to mix for 15 seconds. 9. Centrifuge at 16,000 x *g* for 10 minutes. 10. Transfer **~500 µL aqueous layer (upper)** to a new 1.5 mL microcentrifuge tube (not provided).
Binding	11. Add **600 µL F3 Buffer**. Vortex for 30 seconds to mix. 12. Short spin to bring down the lysate. 13. Place a **PrimeWay Food Column** into a new **Collection Tube**. 14. Transfer **up to 700 µL lysate** into the **PrimeWay Food Column** and centrifuge at 8,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the **Collection Tube**. 15. Repeat Step 14 until all the sample has been transferred to the **PrimeWay Food Column**.
Washing	16. Add **600 µL Wash Buffer FD** to the **PrimeWay Food Column**. Centrifuge at 8,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the **Collection Tube**. 17. Repeat Step 16.

Drying	18. Centrifuge the column at 13,000 x *g* for 3 minutes to dry the column membrane.
Elution	19. Transfer the **PrimeWay Food Column** to a new 1.5 mL microcentrifuge tube (not provided). 20. Add **30 µL preheated Elution Buffer (65 °C)** to the centre of the **PrimeWay Food Column** membrane. Incubate at room temperature for 2 minutes. Centrifuge at 13,000 x *g* for 1 minute to elute the DNA. 21. Store the eluted DNA at 2 – 8 °C or –20 °C for long-term storage.

2. Oil

Materials Supplied by User

- ✓ Vortex mixer
- ✓ Centrifuge, at speed of 8,000 – 14,000 x *g*
- ✓ Water bath or thermoblock
- ✓ Thermomixer (DLAB HM100-Pro LCD digital Thermo Mix, or similar)
- ✓ Cell disruptor (Disruptor Genie, or similar)
- ✓ Hexane
- ✓ 1.5 mL microcentrifuge tube
- ✓ 2 mL microcentrifuge tube
- ✓ 2 mL screw cap tube
- ✓ Pipettes & pipette tips
- ✓ Ice bath

Protocol

Preparation

✓ Preheat the **Elution Buffer** at 65 °C.

Sample	1. Using a pipette, transfer **500 µL oil sample** into a new 2 mL screw cap tube (not provided).
Lysis	2. Add **900 µL F1 Buffer** and **40 µL Proteinase K Solution**. 3. Homogenise the mixture at maximum speed (~2,850 rpm) for 5 minutes using cell disruptor. 4. Incubate the sample in thermomixer at 65 °C with constant shaking (1,000 rpm) for 10 minutes.

Lysis	5. Add **300 µL F2 Buffer**. Vortex to mix for 30 seconds or until homogenised. 6. Incubate the sample on ice (0 – 4 °C) for 5 minutes. 7. Centrifuge at 13,000 x *g* for 5 minutes. 8. Carefully remove the oil layer (top). Using a new pipette tip, pierce through the white interphase and transfer **~700 µL aqueous layer (bottom)** to a new 2 mL microcentrifuge tube (not provided). ***Note:*** *Do not transfer the white interphase.* 9. Add **500 µL hexane** (not provided). Vortex vigorously to mix for 15 seconds. 10. Centrifuge at 14,000 x *g* for 15 minutes. 11. Transfer **~500 µL aqueous layer (bottom)** to a new 1.5 mL microcentrifuge tube.
Binding	12. Add **600 µL F3 Buffer**. Vortex for 30 seconds to mix. Short spin to bring down the lysate. 13. Place a **PrimeWay Food Column** into a new **Collection Tube**. 14. Transfer **up to 700 µL lysate** into the **PrimeWay Food Column** and centrifuge at 8,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the **Collection Tube**. 15. Repeat Step 12 and 14.

Washing	16. Add **600 µL Wash Buffer FD** to the **PrimeWay Food Column**. Centrifuge at 8,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the **Collection Tube**. 17. Repeat Step 16.
Drying	18. Centrifuge the column at 13,000 x *g* for 3 minutes to dry the column membrane.
Elution	19. Transfer the **PrimeWay Food Column** to a new 1.5 mL microcentrifuge tube (not provided). 20. Add **50 µL preheated Elution Buffer (65 °C)** to the centre of the **PrimeWay Food Column** membrane. Incubate at room temperature for 2 minutes. Centrifuge at 13,000 x *g* for 1 minute to elute the DNA. 21. Store the eluted DNA at 2 – 8 °C or −20 °C for long-term storage.

Product Ordering Information

Product Number	Product Description	Remarks
Nacalai Tesque 08401-65	Chloroform, Grade: EP, 500ML	Sufficient for 1000 preps
Nacalai Tesque 17922-65	Hexane, Grade: GR, 500ML	Sufficient for 1000 preps
DLAB Scientific 5062104100	M100-Pro LCD digital Thermo Mix	For sample heating and mixing