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PrimeWay Food DNA Extraction Kit (KIT-9080)

Sample Types

- Solid Food
- Thick/ Concentrated Liquid Food
- Liquid Food
- Liquid Food with Bacteria
- Gel/ Gum/ Gelatine

Molecular Biology Kit





PrimeWay Food DNA Extraction Kit

Product No: KIT-9080

PrimeWay Food DNA Extraction Kit is a rapid and reliable kit designed to isolate DNA from a wide range of food including raw and processed food samples originating from animal, plant, mixed sources, or cultured bacteria. It is suitable for food source authentication and GMO DNA isolation.

This kit utilises a silica-based spin column method, and no hazardous organic solvents (e.g., chloroform) are required. The extracted DNA is free from PCR inhibitors and RNA. It has been proven suitable for downstream applications such as PCR (as short as 90 bp), qPCR, amplicon sequencing, etc.

For Research Use Only. Not for use in Diagnostic Procedures.

Kit Contents

No	Product	KIT-9080-10 10 preps	KIT-9080-50 50 preps
1	F1 Buffer	10 mL	50 mL
2	F2 Buffer	3.2 mL	20 mL
3	F3 Buffer	12 mL	60 mL
4	Wash Buffer FD	4 mL	20 mL
5	Elution Buffer	1.5 mL	20 mL
6	Proteinase K	9 mg	44 mg
7	Proteinase K Buffer	500 µL	3 mL
8	PrimeWay Food Column	10 pcs	50 pcs
9	Collection Tube	10 pcs	50 pcs



Storage

This kit will be delivered at room temperature (21 – 25 °C). Store the kit at room temperature. Reconstituted Proteinase K Solution should be stored at –20 °C.

Product Specification

	KIT-9080
Binding capacity	Up to 100 µg
Sample Size	Refer Table A
Elution	30 – 100 µL
Duration	≤ 30 minutes (exclude lysis incubation)

Sample Material

Table A: Each protocol is optimised with the listed food samples and serves as a guideline to determine which protocol best fits the user's food sample.

Protocol	Input Amount	Food Sample	Page
A (Solid Food)	Up to 200 mg	✓ Canned beef curry	6 – 7
		✓ Canned sardine in tomato sauce	
		✓ Canned tuna in oil	
		✓ Chicken ham	
		✓ Crab stick	
		✓ Flour	
		✓ Macaroni	
		✓ Oat	
		✓ Peanut butter	
		✓ Pollen	
		✓ Popcorn	
		✓ Pork sausage	
		✓ Tofu	
✓ Tuna with chicken in jelly			



Protocol	Input Amount	Food Sample	Page
B (Gel/ Gum/ Gelatine)	50 – 200 mg	<ul style="list-style-type: none"> ✓ Agar ✓ Chewing gum ✓ Gummy candy ✓ Gelatine: <ul style="list-style-type: none"> • Bovine • Porcine • Fish ✓ Gelatine capsule: <ul style="list-style-type: none"> • Bovine • Porcine 	8 – 9
C (Thick/ Concentrated Liquid Food)	i) 500 – 1000 mg ii) 5 mL or 5 g (Jam only)	<ul style="list-style-type: none"> ✓ Tomato puree ✓ Tomato ketchup ✓ Chocolate paste ✓ Jam 	10 – 12
D (Liquid Food)	200 μ L or 200 mg	<ul style="list-style-type: none"> ✓ Soya milk ✓ Dairy products: <ul style="list-style-type: none"> • Cooking cream • Cow milk • Yoghurt (target food origin) 	13 – 14
E (Liquid Food with Bacteria)	i) Up to 1 mL (Liquid food) ii) 200 mg (Yoghurt)	<ul style="list-style-type: none"> ✓ Bacteria culture ✓ Cultured milk ✓ Milk ✓ Yoghurt (target bacteria) 	15 – 17

Note: The complexity and processing of processed foods may vary significantly. Therefore, it is not possible to determine the best protocol for each food sample. Kindly contact us for technical support or any further enquiries.



Materials Supplied by User

- ✓ 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)
- ✓ Absolute ethanol ($\geq 99.5\%$)
- ✓ Ice bath
- ✓ 1.5 mL microcentrifuge tube
- ✓ 2 mL microcentrifuge tube
- ✓ Pipettes & pipette tips
- ✓ Refer **Table B** for the additional materials required for different protocol

Table B: Additional materials supplied by users.

Protocol	Materials
A (Solid Food)	<ul style="list-style-type: none"> ✓ Food processor/ blender ✓ Mortar and pestle ✓ Liquid nitrogen
B (Gel/ Gum/ Gelatine)	<ul style="list-style-type: none"> ✓ Food processor/ blender ✓ Mortar and pestle ✓ Liquid nitrogen
C (Thick/ Concentrated Liquid Food)	<ul style="list-style-type: none"> ✓ Mortar and pestle ✓ Liquid nitrogen ✓ Cell disruptor ✓ 50 mL centrifuge tube (jam only) ✓ 0.1 M Tris Buffer, pH 8.0
D (Liquid Food)	<ul style="list-style-type: none"> ✓ 1 mL syringe
E (Liquid Food with Bacteria)	<ul style="list-style-type: none"> ✓ 1 mL syringe ✓ Bacterial Pre-Lysis Buffer [20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0] ✓ Lysozyme, lyophilised



Precautions for User

- ✓ Some buffers in this kit contain irritants. Handle with care and avoid contact with skin. In case of contact, wash skin with a copious amount of water; seek medical attention.
- ✓ Always wear a lab coat, disposable gloves, and a surgical mask.

Before Start

- ✓ It is highly recommended to read through the manual prior to starting especially for first-time users.
- ✓ Make sure no precipitation is observed in **F1 Buffer** and **F3 Buffer**. Dissolve the precipitate, if any, by incubating the buffer at 60 °C water bath, followed by gentle swirling.
- ✓ Add absolute ethanol ($\geq 99.5\%$) to **Wash Buffer FD** and prepare **Proteinase K Solution** by dissolving **Proteinase K** with **Proteinase K Buffer**.

P/No	Wash Buffer FD (Abs. Ethanol to be added)	Proteinase K (Proteinase K Buffer to be added)
KIT-9080-10	16 mL	450 μL *
KIT-9080-50	80 mL	2.2 mL*

* After reconstitution, store Proteinase K Solution at $-20\text{ }^{\circ}\text{C}$.

Sample Homogenisation

Pre-cool the **mortar and pestle** using liquid nitrogen (LN_2). Freeze the food samples immediately in LN_2 and grind the sample into a fine powder under LN_2 . Keep the sample frozen throughout the grinding process. Transfer the ground sample into a 2 mL microcentrifuge tube. Snap freezing the sample in LN_2 and store the samples in $-80\text{ }^{\circ}\text{C}$. The samples are stable up to 6 months.



Protocol A – Solid Food

Preparation

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

Sample	<ol style="list-style-type: none"> 1. Homogenise food sample as finely as possible using a blender. 2. [Optional] Further grind the food sample into fine powder using mortar and pestle with liquid nitrogen. <i>Refer page 5 for details of Sample Homogenisation.</i> 3. Transfer up to 200 mg homogenised food sample into a new 2 mL microcentrifuge tube (not provided).
Lysis	<ol style="list-style-type: none"> 4. Add 900 µL F1 Buffer and 20 µL Proteinase K Solution. Vortex to mix for 30 seconds or until homogenised. 5. Incubate the sample in thermomixer at 65 °C with constant shaking (1,000 rpm) for 60 minutes. 6. Add 300 µL F2 Buffer. Vortex to mix for 30 seconds or until homogenised. 7. Incubate the sample on ice (0 – 4 °C) for 5 minutes. 8. Centrifuge at 13,000 x g for 5 minutes. 9. Transfer 400 – 600 µL clear supernatant to a new 1.5 mL microcentrifuge tube (not provided). Note: <i>If a floating layer/film forms on top of the supernatant, pierce through the layer and pipette only the clear solution.</i>



Binding	<p>10. Add 600 μL F3 Buffer. Vortex for 30 seconds to mix.</p> <p>11. Short spin to bring down the lysate.</p> <p>12. Place a PrimeWay Food Column into a new Collection Tube.</p> <p>13. Transfer up to 700 μL lysate into the PrimeWay Food Column and centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>14. Repeat Step 13 until all the sample has been transferred to the PrimeWay Food Column.</p>
Washing	<p>15. Add 600 μL Wash Buffer FD to the PrimeWay Food Column. Centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>16. Repeat Step 15.</p>
Drying	<p>17. Centrifuge the column at 13,000 x <i>g</i> for 3 minutes to dry the column membrane.</p>
Elution	<p>18. Transfer the PrimeWay Food Column to a new 1.5 mL microcentrifuge tube (not provided).</p> <p>19. 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 <i>g</i> for 1 minute to elute the DNA. <i>Note: If expected DNA yield is less than 2 μg, reduce Elution Buffer volume to 30 μL.</i></p> <p>20. [Optional] For maximum recovery, repeat Step 19 with a new 1.5 mL microcentrifuge tube (not provided).</p> <p>21. Store the eluted DNA at 2 – 8 °C or –20 °C for long-term storage.</p>



Protocol B – Gel/ Gum/ Gelatine

Preparation

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

Sample	<ol style="list-style-type: none"> 1. Homogenise food sample as finely as possible using a blender. 2. [Optional] Further grind the food sample into fine powder using mortar and pestle with liquid nitrogen. <i>Refer page 5 for details of Sample Homogenisation.</i> 3. Transfer 50 – 200 mg homogenised food sample into a new 2 mL microcentrifuge tube (not provided).
Lysis	<ol style="list-style-type: none"> 4. Add 900 µL F1 Buffer and 20 µL Proteinase K Solution. Vortex to mix for 30 seconds or until homogenised. 5. Incubate the sample in thermomixer at 65 °C with constant shaking (1,000 rpm) for 15 minutes. Note: <i>After incubation, if the mixture formed solid, reduce the starting material to 50 mg.</i> 6. Add 300 µL F2 Buffer. Vortex to mix for 30 seconds or until homogenised. 7. Incubate the sample on ice (0 – 4 °C) for 5 minutes. 8. Centrifuge at 13,000 x g for 5 minutes. 9. Transfer 400 – 600 µL clear supernatant to a new 1.5 mL microcentrifuge tube (not provided). Note: <i>If precipitates are observed in the supernatant, repeat Step 8 and 9.</i>



Binding	<p>10. Add 600 µL F3 Buffer. Vortex for 30 seconds to mix.</p> <p>11. Short spin to bring down the lysate.</p> <p>12. Place a PrimeWay Food Column into a new Collection Tube.</p> <p>13. Transfer up to 700 µL lysate into the PrimeWay Food Column and centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>14. Repeat Step 13 until all the sample has been transferred to the PrimeWay Food Column.</p>
Washing	<p>15. Add 600 µL Wash Buffer FD to the PrimeWay Food Column. Centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>16. Repeat Step 15.</p>
Drying	<p>17. Centrifuge the column at 13,000 x <i>g</i> for 3 minutes to dry the column membrane.</p>
Elution	<p>18. Transfer the PrimeWay Food Column to a new 1.5 mL microcentrifuge tube (not provided).</p> <p>19. 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 <i>g</i> for 1 minute to elute the DNA.</p> <p>20. Store the eluted DNA at 2 – 8 °C or –20 °C for long-term storage.</p>



Protocol C – Thick/ Concentrated Liquid Food

Materials Supplied by User

- ✓ 0.1 M Tris Buffer, pH 8.0
- ✓ [Optional] pH strip

Preparation

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

Sample

1A) Thick/ concentrated liquid food

- Transfer **500 – 1000 mg food sample** into a new 2 mL microcentrifuge tube (not provided).
- Add **1 mL of 0.1 M Tris Buffer** (not provided) to wash the sample. Homogenise the mixture using a cell disruptor at maximum speed (~3,000 rpm) for 2 minutes.
- Centrifuge at maximum speed (~16,000 x g) for 1 minute. Discard the supernatant.
- Repeat Step ii and iii with the total washing of two times.
- [Optional] Examine the supernatant with pH strip. The ideal pH is 6.0 – 8.0.

OR

1B) For Jam ONLY

- Transfer **5 mL or 5 g food sample** into a new 50 mL centrifuge tube (not provided).
- Add **5 mL of 0.1 M Tris Buffer** (not provided) to wash the sample. Vortex to mix until homogenised.
- Centrifuge at 2,500 x g for 5 minutes. Discard the supernatant.
- Repeat Step ii and iii with the total washing of two times.



Sample	<p>v) [Optional] Examine supernatant with pH strip. The ideal pH is pH 6.0 – 8.0.</p> <p>vi) Transfer the pellet into mortar and pestle. Grind the pellet into fine powder with liquid nitrogen. <i>Refer page 5 for details of Sample Homogenisation.</i></p> <p>vii) Weigh 500 mg ground sample into a new 2 mL microcentrifuge tube (not provided).</p>
Lysis	<p>2. Add 900 µL F1 Buffer and 40 µL Proteinase K Solution. Vortex to mix for 30 seconds or until homogenised.</p> <p>3. Incubate the sample in thermomixer at 65 °C with constant shaking (1,000 rpm) for the duration as below:</p> <p>i) Thick/ concentrated liquid food: 15 minutes.</p> <div style="text-align: center; border: 1px solid black; padding: 5px; width: fit-content; margin: 10px auto;"> OR </div> <p>ii) Jam ONLY: 15 minutes to overnight.</p> <p>4. Centrifuge at maximum speed (~16,000 x g) for 1 minute.</p> <p>5. Transfer 900 µL supernatant to a new 2 mL microcentrifuge tube (not provided).</p> <p>6. Add 300 µL F2 Buffer. Vortex to mix for 30 seconds or until homogenised.</p> <p>7. Incubate the sample on ice (0 – 4 °C) for 5 minutes.</p> <p>8. Centrifuge at 13,000 x g for 5 minutes.</p> <p>9. Transfer 400 – 600 µL clear supernatant to a new 1.5 mL microcentrifuge tube (not provided).</p>



Binding	<p>10. Add 600 μL F3 Buffer. Vortex for 30 seconds to mix.</p> <p>11. Short spin to bring down the lysate.</p> <p>12. Place a PrimeWay Food Column into a new Collection Tube.</p> <p>13. Transfer up to 700 μL lysate into the PrimeWay Food Column and centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>14. Repeat Step 13 until all the sample has been transferred to the PrimeWay Food Column.</p> <p>15. Add 500 μL F3 Buffer to the PrimeWay Food Column.</p> <p>16. Centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p>
Washing	<p>17. Add 600 μL Wash Buffer FD to the PrimeWay Food Column. Centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>18. Repeat Step 17.</p>
Drying	<p>19. Centrifuge the column at 13,000 x <i>g</i> for 3 minutes to dry the column membrane.</p>
Elution	<p>20. Transfer the PrimeWay Food Column to a new 1.5 mL microcentrifuge tube (not provided).</p> <p>21. 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 <i>g</i> for 1 minute to elute the DNA.</p> <p>22. Store the eluted DNA at 2 – 8 °C or –20 °C for long-term storage.</p>



Protocol D – Liquid Food

Materials Supplied by User

- ✓ 1 mL syringe

Preparation

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

Sample	<ol style="list-style-type: none"> 1. Using a syringe (not provided), transfer 200 µL or 200 mg liquid food sample into a new 2 mL microcentrifuge tube (not provided).
Lysis	<ol style="list-style-type: none"> 2. Add 900 µL F1 Buffer and 20 µL Proteinase K Solution. Vortex 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. 5. Incubate the sample on ice (0 – 4 °C) for 5 minutes. 6. Centrifuge at 13,000 x g for 5 minutes. 7. Transfer 400 – 600 µL clear supernatant to a new 1.5 mL microcentrifuge tube (not provided). Note: <i>If a floating layer/ film forms on top of the supernatant, pierce through the layer and pipette only the clear solution.</i>



Binding	<p>8. Add 600 μL F3 Buffer. Vortex for 30 seconds to mix.</p> <p>9. Short spin to bring down the lysate.</p> <p>10. Place a PrimeWay Food Column into a new Collection Tube.</p> <p>11. Transfer up to 700 μL lysate into the PrimeWay Food Column and centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>12. Repeat Step 11 until all the sample has been transferred to the PrimeWay Food Column.</p>
Washing	<p>13. Add 600 μL Wash Buffer FD to the PrimeWay Food Column. Centrifuge at 8,000 x <i>g</i> for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>14. Repeat Step 13.</p>
Drying	<p>15. Centrifuge the column at 13,000 x <i>g</i> for 3 minutes to dry the column membrane.</p>
Elution	<p>16. Transfer the PrimeWay Food Column to a new 1.5 mL microcentrifuge tube (not provided).</p> <p>17. 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 <i>g</i> for 1 minute to elute the DNA.</p> <p>18. Store the eluted DNA at 2 – 8 °C or –20 °C for long-term storage.</p>



Protocol E – Liquid Food with Bacteria

Reagents Supplied by User

- ✓ Bacterial Pre-Lysis Buffer [20 mM Tris-HCl; 2 mM EDTA; 1% Triton X-100; pH 8.0]
- ✓ Lysozyme, lyophilised
- ✓ 1 mL syringe

Preparation

- ✓ Preheat the **Elution Buffer** at 65 °C.
- ✓ **Bacterial Pre-Lysis Buffer Mix preparation:**
Dissolve **20 mg lysozyme** (not provided) into **1 mL Bacterial Pre-Lysis Buffer** (not provided).

Sample	1A) <u>Bacterial Culture</u> i) Transfer up to 1 mL overnight bacterial culture into a new 2 mL microcentrifuge tube (not provided). ii) Centrifuge at maximum speed ($\sim 16,000 \times g$) for 3 minutes. Discard the supernatant.
	OR
	1B) <u>Milk/ Cultured Milk</u> i) Using a syringe, transfer up to 1 mL food sample into a new 2 mL microcentrifuge tube (not provided). ii) Centrifuge at maximum speed ($\sim 16,000 \times g$) for 3 minutes. Discard the supernatant, including any creamy layer floating on top. Use a cotton bud to remove any creamy residue adhering to the tube wall.
	OR
	1C) <u>Yoghurt</u> Using a syringe, transfer 200 mg yoghurt into a new 2 mL microcentrifuge tube (not provided).



Lysis

2. Add **180 μL freshly prepared Bacterial Pre-Lysis Buffer Mix** into the sample. Vortex to mix until homogenised.
3. Incubate the sample in thermomixer at $37\text{ }^{\circ}\text{C}$ with constant shaking (1,000 rpm) for 30 minutes.
4. Add **900 μL F1 Buffer** and **20 μL Proteinase K Solution**. Vortex to mix for 30 seconds or until homogenised.
5. Incubate the sample in thermomixer at $65\text{ }^{\circ}\text{C}$ with constant shaking (1,000 rpm) for 60 minutes.
6. Add **300 μL F2 Buffer**. Vortex to mix for 30 seconds or until homogenised.
7. Incubate the sample on ice ($0 - 4\text{ }^{\circ}\text{C}$) for 5 minutes.
8. Centrifuge at $13,000 \times g$ for 5 minutes.
9. Transfer **400 – 600 μL clear supernatant** to a new 1.5 mL microcentrifuge tube (not provided).
Note: If precipitates are observed in the supernatant, repeat Step 8 and 9.

Binding

10. Add **600 μL F3 Buffer**. Vortex for 30 seconds to mix.
11. Short spin to bring down the lysate.
12. Place a **PrimeWay Food Column** into a new **Collection Tube**.
13. Transfer **up to 700 μL lysate** into the **PrimeWay Food Column** and centrifuge at $8,000 \times g$ for 30 seconds. Discard the flow-through and place the column back into the **Collection Tube**.
14. Repeat Step 13 until all the sample has been transferred to the **PrimeWay Food Column**.



Washing	<p>15. Add 600 μL Wash Buffer FD to the PrimeWay Food Column. Centrifuge at $8,000 \times g$ for 30 seconds. Discard the flow-through and place the column back into the Collection Tube.</p> <p>16. Repeat Step 15.</p>
Drying	<p>17. Centrifuge the column at $13,000 \times g$ for 3 minutes to dry the column membrane.</p>
Elution	<p>18. Transfer the PrimeWay Food Column to a new 1.5 mL microcentrifuge tube (not provided).</p> <p>19. Add 50 – 100 μL preheated Elution Buffer ($65\text{ }^\circ\text{C}$) to the centre of the PrimeWay Food Column membrane. Incubate at room temperature for 2 minutes. Centrifuge at $13,000 \times g$ for 1 minute to elute the DNA.</p> <p>20. [Optional] For maximum recovery, repeat Step 19 with a new 1.5 mL microcentrifuge tube (not provided).</p> <p>21. Store the eluted DNA at $2 - 8\text{ }^\circ\text{C}$ or $-20\text{ }^\circ\text{C}$ for long-term storage.</p>



Troubleshooting Guidelines

The DNA extracted from the processed food is typically degraded and/or present in low amounts. PCR or qPCR is required to verify the presence of target DNA. If the results are not as expected, refer to the troubleshooting guidelines as below.

Problems	Possible Reason	Recommended Action
Low DNA Yield	Poor homogenisation of sample	After grinding the sample with blender, further grind the sample to fine powder using LN ₂ or bead mills. Refer to page 5 “Sample Homogenisation” for LN ₂ grinding method in detail.
	Nature of the sample	Increase the starting material.
		Increase the input of Proteinase K Solution to 40 µL.
	Insufficient lysis	Increase the incubation time after adding F1 Buffer and Proteinase K Solution to 60 minutes.
	Clogged column	Do not exceed the recommended amount of starting sample.
		Do not transfer any debris while transferring supernatant. Repeat centrifugation if floating debris is observed in the supernatant.
	Sample remain acidic (Thick/ concentrated liquid food)	After centrifugation in “Sample” step, check the pH of supernatant with pH strip, the ideal pH is pH 6.0 – 8.0. If the pH is less than 6, repeat washing with 0.1 M Tris Buffer until the pH value falls within the ideal range.
Inappropriate buffer preparation	Ensure the preparation of Wash Buffer FD and Proteinase K Solution is according to the protocol in “Before Start”, Page 5.	
Did not preheat the Elution Buffer	Ensure Elution Buffer is preheated to 65 °C before elution. Ensure the Elution Buffer is completely absorbed by the membrane.	
Precipitate not observed after centrifugation in “Lysis” step	Incorrect centrifugation condition	Repeat vortexing until homogenised. Centrifuge at 13,000 x g for 5 minutes.



Problems	Possible Reason	Recommended Action
Gel formed after adding F1 buffer (sample that swell greatly)	Too much sample input	Do not use sample input more than 50 mg. Determine the sample input empirically. Checkpoint: After incubation at 65 °C, the lysate should remain in liquid form. It is recommended to recover 400 – 600 µL of supernatant after centrifugation.
Debris remain in supernatant after centrifugation in “Lysis” step	Insufficient centrifugation time/ nature of the sample	Transfer the supernatant into a new 1.5 mL microcentrifuge tube. Repeat centrifugation at 13,000 x <i>g</i> for 5 minutes. Transfer the clear supernatant into a new 1.5 mL microcentrifuge tube and proceed to “Binding” step.
Bad A260/280 ratio	Complex food samples	A260/A280 ratio does not accurately represent DNA quality for food samples as traces of food matrix in the sample will affect the spectroscopic reading.
Downstream Applications		
No PCR product	Amplicon size is large (> 250 bp)	The gDNA may be too degraded. Reduce targeted amplicon size to 90 – 150 bp.
	Low gDNA template	Perform PCR optimisation. The volume of gDNA as PCR template can be increased up to 50% of the PCR reaction mix.
		The DNA amount is too low. Use 5 µL of the first PCR product as template to perform second PCR to verify the presence of the target DNA.
	Low PCR sensitivity	Target different gene region or design a new primer pair.
Smearing of target PCR band	Prolonged lysis time	Processed food contains highly fragmented DNA. Long lysis time might cause further DNA degradation. Reduce the lysis time from 60 minutes to 15 minutes at 65 °C.

Please contact us at <https://base-asia.com/contact/> for more information.



Product Ordering Information

Product Number	Product Description	Remarks
8031122000	MX-C Cell disruptor	For sample homogenisation.
5062104100	HM100-Pro LCD digital Thermo Mix with heating, mixing	For sample incubation and mixing.
BUF-1416-500ml-pH8.0	1.0M Tris Buffer, pH 8.0, Biotechnology Grade, 500ml	Dilute to 0.1 M Tris Buffer. Sufficient for at least 500 preps.
K.BUF-9105-50ml	Bacterial Pre-Lysis Buffer, 50mL	Sufficient for up to 270 preps.
K.RGT-9108-110mg	Lysozyme, 110 mg/vial	Sufficient for 25 preps.

