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PrimeWay Stool DNA Extraction Kit (KIT-9070)

Molecular Biology Kit

PrimeWay Stool DNA Extraction Kit

Product No: KIT-9070

PrimeWay Stool DNA Extraction Kit is a robust and reliable kit designed to isolate genomic DNA from both animal and human stool sample, as well as gut content. This kit is meticulously optimised to handle broad spectrum of stool types such as normal, dry, hard & high fibre stool. Ceramic beads coupled with homogenisation duration is perfected in this kit to maximise the lysis of the microorganism. The extracted DNA is free from PCR inhibitor, making it suitable for downstream application such as PCR, amplicon sequencing, qPCR, Southern blot, etc.

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

Kit Contents

No	Product	KIT-9070-10 10 preps	KIT-9070-50 50 preps
1	STL1 Buffer	9 mL	50 mL
2	STL2 Buffer	1.8 mL	15 mL
3	STL3 Buffer	14 mL	2 x 45 mL
4	Wash Buffer ST	3 mL	25 mL
5	Elution Buffer	1.5 mL	6 mL
6	Inhibitor Removal Column	10 pcs	50 pcs
7	PrimeWay Stool Column	10 pcs	50 pcs
8	Stool Bead Tube	10 pcs	50 pcs
9	2 mL Microcentrifuge Tube	10 pcs	50 pcs
10	Collection Tube	10 pcs	50 pcs

Storage

This kit can be stored at room temperature (21 – 25 °C).

Product Specification

	KIT-9070
Binding capacity	Up to 50 µg
Sample Size	Refer "Sample Material"
Elution	30 – 100 µL
Duration	≤ 60 minutes

Sample Material

- ✓ Sample types:

	Input Amount	Example
Human stool (soft)	180 – 220 mg	Human
Animal stool (soft) or gut content	60 – 100 mg	Dog, cat, mouse gut content
Dry, hard or high fibre animal stool	60 – 100 mg	Horse, rabbit, mouse

- ✓ Upon stool collection, stool samples should be kept at 2 – 8 °C if the extraction process is intended to be carried out on the same day. If DNA extraction is not performed on the same day, store the stool in –80 °C until further processing.
- ✓ Frozen stool is required to be thawed and kept on ice before DNA extraction.

Materials Supplied by User

- ✓ Absolute ethanol (≥ 99.5 %)
- ✓ Centrifuge, at speed of 4,000 – 16,000 x *g*
- ✓ 1.5 mL microcentrifuge tubes
- ✓ 2 mL microcentrifuge tube
- ✓ 50 centrifuge tube (for hard, dry or high fibre animal stool only)
- ✓ Pipettes & pipette tips

- ✓ Vortex mixer
- ✓ Cell Disruptor (Digital Disruptor Genie, or similar instrument)
- ✓ Water bath
- ✓ Blender (for hard, dry or high fibre animal stool only)

Precautions for Use

- ✓ Stool samples should be handled in Laboratory Biosafety Level 2 (BSL-2) as it may contain pathogens such as bacteria, fungus, virus and parasite.
- ✓ Always wear a lab coat, disposable gloves, and surgical mask.
- ✓ 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.

Before Start

- ✓ It is highly recommended to read through the manual prior to starting, especially for a first-time user.
- ✓ Ensure that no precipitation is observed in **STL1 Buffer**. If any precipitate is present, dissolve it by incubating the buffer at 37 °C water bath, followed by gently shaking.
- ✓ Add absolute ethanol (≥ 99.5 %) to Wash Buffer ST as below:

P/No	Ethanol to be added
KIT-9070-10	12 mL
KIT-9070-50	100 mL

Protocol

Reagent Supplied by User

- ✓ [Hard, dry or high fibre animal stool only] 1X Tris-EDTA (TE) Buffer, pH 8.0
- ✓ [Optional] 100 mg/ mL RNase A

Preparation

I. Preheat the **Elution Buffer** at 60 °C.

Sample

1A) Human stool (soft)

i) Transfer **180 – 220 mg stool** into the **Stool Bead Tube**. Proceed to Step 2.

OR

1B) Animal stool (soft) or gut content

Transfer **60 – 100 mg stool or gut content** into the **Stool Bead Tube**. Proceed to Step 2.

OR

1C) Hard, dry or high fibre animal stool

i) Add 1 volume of stool with 5 volumes of 1X TE, pH 8 into the blender.

ii) Blend the stool with pulse mode until homogenise. This is to break the dry stool and/or fibre break into pieces and ensure uniform distribution of microbiota.

iii) Transfer the stool mixture into a new 50 mL centrifuge tube (not provided).

iv) Centrifuge at 4,000 x *g* for 5 minutes. Discard supernatant.

v) Weight **60 – 100 mg stool** into the **Stool Bead Tube**.

vi) Proceed to Step 2.

Note: DO NOT transfer undigested food such as crop, fruit husks and undigested seeds into the **Stool Bead Tube**.

Lysis	2. Add **800 µL STL1 Buffer** into the **Stool Bead Tube**. 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. 8. Incubate on ice (0 – 4 °C) for 5 minutes. 9. Centrifuge at 16,000 x *g* for 3 minutes.
Inhibitor Removal	10. Place an **Inhibitor Removal Column** (purple ring) into a **2 mL Microcentrifuge Tube**. 11. Transfer **500 µL clear supernatant** into the **Inhibitor Removal Column** and centrifuge at 16,000 x *g* for 1 minute. 12. KEEP THE FLOW-THROUGH and discard the column. ***Note***: If pellet is observed in the flow-through, transfer the clear supernatant to a new 2 mL microcentrifuge tube (not provided). 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.

Binding	14. Add **800 µL STL3 Buffer** to the flow-through and immediately shake vigorously for 5 seconds. 15. Short spin to bring down the lysate. 16. Place a **PrimeWay Stool Column** (green ring) into a new **Collection Tube**. 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**. 18. Repeat Step 17 until all the lysate has been transferred to the **PrimeWay Stool Column**.
Washing	19. Add **400 µL STL3 Buffer** to the **PrimeWay Stool Column**. Centrifuge at 16,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the **Collection Tube**. 20. Add **600 µL Wash Buffer ST** to the **PrimeWay Stool Column**. Centrifuge at 16,000 x *g* for 30 seconds. Discard the flow-through and place the column back into the **Collection Tube**. 21. Repeat Step 20.
Drying	22. Centrifuge the column at 16,000 x *g* for 3 minute to dry the column membrane.

Elution	
	23. Transfer the **PrimeWay Stool Column** to a new 1.5 mL microcentrifuge tube (not provided). 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 *g* for 2 minutes to elute the DNA. 25. Store the eluted DNA at 2 – 8 °C or –20 °C for long-term storage.

Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low DNA Yield or no recovery	Too many sample	Too little space available for beads beating. Use the recommended/ lesser amount of stool.
	Did not preheat the Elution Buffer	Preheat the Elution Buffer at 60 °C before Elution Step. Ensure the Elution Buffer is completely absorbed by the membrane.
	Inappropriate buffer preparation	Ensure the correct amount of absolute ethanol is added to the Wash Buffer ST before use.
		Ensure no precipitation formed in STL1 Buffer. Dissolve the precipitate by incubating in a 37 °C water bath, followed by gentle shaking.
Degraded DNA	Inappropriate storage condition	Upon stool collection, stool samples should be kept at 2 – 8 °C if the extraction process is intended to be carried out on the same day. If DNA extraction is not performed on the same day, store the stool in –20 °C until further processing.
	Harsh mechanical disruption	Reduce disruption (homogenisation) speed and/ or time.
No PCR amplification	Presence of PCR inhibitors	Dilute the DNA to reduce concentration of PCR inhibitors.
DNA floats out of agarose gel well during loading	Presence of ethanol residual	In the Drying step, extend the centrifugation time to 5 minutes to ensure the PrimeWay Stool Column is completely dry.

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

Product Ordering Information

Product Number	Product Description	Remarks
BUF-3024-1X100ml	1X Tris-EDTA (TE) Buffer, pH 8.0, Biotechnology Grade, 100ml	For dry, hard or high fibre stool only.
K.RGT-9110-1ml	RNase A Solution, 100mg/mL, 1mL	Optional if RNA-free DNA is required.