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PrimeWay Soil DNA Extraction Kit (KIT-9060)

Sample Types

■ Soil ■ Manure ■ Water

Molecular Biology Kit

PrimeWay Soil DNA Extraction Kit

Product No: KIT-9060

PrimeWay Soil DNA Extraction Kit is a reliable kit that is used to isolate genomic DNA from soil sample. Both mechanical and chemical lysis methods are used for maximum extraction efficiency and DNA yield. This kit can efficiently remove abundance of humic substances and pigments which affect downstream processes such as PCR. Besides soil sample, it is also suitable for other sample types including animal manure, worm compost and water. The purified DNA is suitable for PCR, Southern blot, enzyme digestion, amplicon sequencing, etc.

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

Kit Contents

No	Product	KIT-9060-10 10 preps	KIT-9060-50 50 preps	KIT-9060-250 250 preps
1	SL1 Buffer	9 mL	60 mL	250 mL
2	SL2 Buffer	0.8 mL	5 mL	20 mL
3	SL3 Buffer	1.8 mL	10 mL	50 mL
4	SIR Buffer	1.8 mL	10 mL	2 x 25 mL
5	SBD Buffer	9 mL	40 mL	220 mL
6	Wash Buffer S1	4.2 mL	21 mL	105 mL
7	Wash Buffer S2	3 mL	20 mL	2 x 50 mL
8	Elution Buffer	1.2 mL	15 mL	30 mL
9	PrimeWay Soil Column	10 pcs	50 pcs	5 x 50 pcs
10	Collection Tube	10 pcs	50 pcs	5 x 50 pcs
11	Soil Bead Tube	10 pcs	50 pcs	5 x 50 pcs

Storage

This kit will be delivered in room temperature (21 – 25 °C). **Upon receipt of the kit, remove the SIR Buffer and store it at 2 - 8 °C.** Short-term storage of the SIR Buffer at room temperature (15 – 25 °C) can be stable up to 24 weeks without affecting the performance. The remaining products of the kit can be stored at room temperature (15 – 25 °C).

Product Specification

	KIT-9060
Binding capacity	100 µg
Sample Size	200 – 500 mg
Elution	50 µL
Duration	≤ 60 minutes

Sample Material

- ✓ Sample input amount:

Protocol	Sample Type	Input amount	Page
A	Soil	200 – 500 mg	5 – 7
B	Manure	100 – 500 mg	8 – 10
C	Water	300 – 500 mL	11 – 13
Clean-up	DNA	Up to 300 µL	14 – 15

- ✓ Do not exceed the 1 mL mark when the sample material (including the glass beads) is filled into the Soil Bead Tube to ensure efficient cell disruption.
- ✓ Remove foreign object such as stone, twigs, root, or bark.
- ✓ If the sample contains high amount of water, bring down the sediment by centrifuge and remove the supernatant. Proceed to Protocol A.
- ✓ If the sample is expected to have very low DNA content (e.g., clay soil), it is not recommended to use SIR Buffer. Skip Step 8 & 9 in the

Inhibitor Removal section. This buffer is effective for pigments or other inhibitors absorption, but it will also reduce the DNA yield.

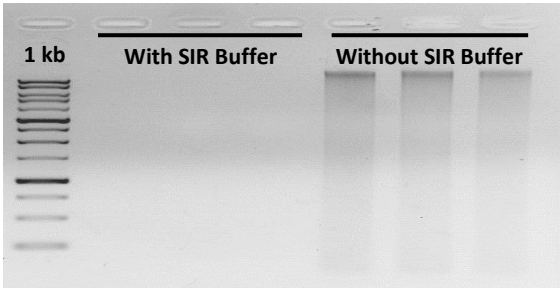

Figure 1: DNA is extracted from 200 mg of clay soil, with and without SIR Buffer, eluted with 50 µL Elution Buffer. 10 µL of the eluted DNA are analysed on 1% agarose gel.

Table A: *The effects of SIR Buffer on clay soil with very low DNA content.*

SIR Buffer	With	Without
Average yield (µg)	0.11	0.35
Average A_{260}/A_{280}	0.33	1.88

Materials Supplied by User

- ✓ Absolute ethanol (≥ 99.5 %)
- ✓ Isopropanol
- ✓ Centrifuge, at speed of 10,000 – 13,000 x g
- ✓ 1.5 mL microcentrifuge tubes
- ✓ 2 mL microcentrifuge tube
- ✓ Pipettes & pipette tips
- ✓ Wide bore tips
- ✓ Vortex mixer
- ✓ Cell Disruptor (DLab MX-C Cell Disruptor, Digital Disruptor Genie, or similar instrument)
- ✓ Water bath

- ✓ 25 mm membrane filter, 0.22 µm or 0.45 µm pore size (Water sample only)
- ✓ 100 µm membrane filter (Optional for water sample only)

Precautions for Use

- ✓ 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 surgical mask.

Before Start

- ✓ It is highly recommended to read through the manual prior start especially for first-time user.
- ✓ Make sure no precipitation observed in **SL2 Buffer** and **SBD Buffer**. Dissolve the precipitate if any, by incubating the buffer at 55 °C water bath before use.
- ✓ Add isopropanol to Wash Buffer S1 and absolute ethanol (≥ 99.5 %) to Wash Buffer S2 as below:

P/No	Wash Buffer S1 (Isopropanol to be added)	Wash Buffer S2 (Abs. Ethanol to be added)
KIT-9060-10	1.8 mL	12 mL
KIT-9060-50	9 mL	80 mL
KIT-9060-250	45 mL	200 mL

Mechanical Disruption of Sample

Table B: *Disruptor setting.*

Disruptor	Speed	Time
Analog (e.g., DLAB MX-C Cell Disruptor)	Max (2500 rpm)	20 minutes
Digital (e.g., Digital Disruptor Genie)	Max (2850 rpm)	20 minutes

Protocol A – Soil

Preparation	I. Allow the SIR Buffer from 4 °C equilibrate to room temperature for 30 minutes before use. II. Preheat the **Elution Buffer** at 65 °C.
Sample	1. Transfer 200 – 500 mg soil sample into the **Soil Bead Tube**. ***Note:*** *Do not exceed the 1 mL mark when filling the sample (including the glass beads) into the tube.*
Lysis	2. Add **800 µL SL1 Buffer** and **60 µL SL2 Buffer** into the **Soil Bead Tube**. 3. Disrupt the sample with max speed for 20 minutes. See Mechanical Disruption of Sample, page 4 for more information. 4. Short spin for 5 seconds to bring down the mixture and incubate at 65 °C (water bath) for 10 minutes. 5. Centrifuge at 13,000 x *g* for 1 minute. 6. Transfer **600 µL supernatant** into a new 1.5 mL microcentrifuge tube. 7. Add **150 µL SL3** Buffer and vortex to mix.

Inhibitor Removal	8. Shake the **SIR Buffer** to ensure content is homogenized. With a wide bore tip, **add 150 µL SIR Buffe**r to the mixture. 9. Vortex to mix and incubate for 3 minutes at room temperature. 10. Centrifuge at maximum speed (≥ 13,000 x *g*) for 5 minutes. 11. Transfer the clear supernatant (~ 550 – 750 µL) to a new 2 mL microcentrifuge tube.
Binding	12. Add **equal volume of SBD Buffer (~ 550 – 750 µL)** and mix by inverting the tube 4 – 6 times. 13. Short spin to bring down the lysate. 14. Place a **PrimeWay Soil Column** into a new **Collection Tube**. 15. Transfer **up to 750 µL lysate** into the **PrimeWay Soil Column** and centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 16. Repeat Step 15 until all the sample has been transferred to the **PrimeWay Soil Column**.
Washing	17. Add **500 µL Wash Buffer S1** to the **PrimeWay Soil Column** and incubate for 1 minute. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 18. Add **650 µL Wash Buffer S2** to the **PrimeWay Soil Column**. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 19. Repeat Step 18.

Drying	20. Centrifuge the column at 10,000 x *g* for 1 minute to dry the column membrane.
Elution	21. Transfer the **PrimeWay Soil Column** to a new 1.5 mL microcentrifuge tube. 22. Add **50 µL preheated Elution Buffer (65 °C)** to the center of the **PrimeWay Soil Column** membrane. Incubate at room temperature for 3 minutes. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA. 23. [Optional] For maximum recovery, repeat Step 22 with a new 1.5 mL microcentrifuge tube. 24. Store the eluted DNA at 2 – 8 °C or -20 °C for long-term storage.

Protocol B – Manure

Preparation	I. Allow the SIR Buffer from 4 °C equilibrate to room temperature for 30 minutes before use. II. Preheat the **Elution buffer** at 65 °C.
Sample	1. Transfer 100 – 500 mg manure sample into the **Soil Bead tube**. ***Note:*** *Do not exceed the 1 mL mark when filling the sample (including the glass beads) into the tube.*
Lysis	2. Add **800 µL SL1 Buffer** and **60 µL SL2 Buffer** into the **Soil Bead Tube**. 3. Homogenize the sample with max speed for 20 minutes. See Mechanical Disruption of Sample, page 4 for more information. 4. Short spin for 5 seconds to bring down the mixture and incubate at 65 °C (water bath) for 10 minutes. 5. Centrifuge at 13,000 x *g* for 1 minute. 6. Transfer **600 µL supernatant** into a new 1.5 mL microcentrifuge tube. 7. Add **150 µL SL3** Buffer and vortex to mix.

Inhibitor Removal	8. Shake the **SIR Buffer** to ensure content is homogenized. With a wide bore tip, **add 150 µL SIR Buffe**r to the mixture. 9. Vortex to mix and incubate for 3 minutes at room temperature. 10. Centrifuge at maximum speed (≥ 13,000 x *g*) for 5 minutes. 11. Transfer the clear supernatant (~ 550 – 750 µL) to a new 2 mL microcentrifuge tube. ***Note:*** *[Optional] When maximum input of manure is used, the supernatant may remain coloured. Repeat Step 8 – 11.*
Binding	12. Add **equal volume of SBD Buffer (~ 550 – 750 µL)** and mix by inverting the tube 4 – 6 times. 13. Short spin to bring down the lysate. 14. Place a **PrimeWay Soil Column** into a new **Collection Tube**. 15. Transfer **up to 750 µL lysate** into the **PrimeWay Soil Column** and centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 16. Repeat Step 15 until all the sample has been transferred to the **PrimeWay Soil Column**.
Washing	17. Add **500 µL Wash Buffer S1** to the **PrimeWay Soil Column** and incubate for 1 minute. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 18. Add **650 µL Wash Buffer S2** to the **PrimeWay Soil Column**. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 19. Repeat Step 18.

Drying	20. Centrifuge the column at 10,000 x *g* for 1 minute to dry the column membrane.
Elution	21. Transfer the **PrimeWay Soil Column** to a new 1.5 mL microcentrifuge tube. 22. Add **50 µL preheated Elution Buffer (65 °C)** to the center of the **PrimeWay Soil Column** membrane. Incubate at room temperature for 3 minutes. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA. 23. [Optional] For maximum recovery, repeat Step 22 with a new 1.5 mL microcentrifuge tube. 24. Store the eluted DNA at 2 – 8 °C or -20 °C for long-term storage.

Protocol C – Water Sample

Materials Supplied by User

- ✓ 100 µm cell strainer (optional)
- ✓ 0.22 µm or 0.45 µm membrane filter, 25 mm
- ✓ Vacuum filtration system

Preparation	I. Preheat the **Elution buffer** at 65 °C.
Sample	1. [Optional] Pre-filter the water sample with bigger membrane filter pore size (e.g., 100 µm) to remove foreign materials and sediment. 2. Perform vacuum filtration with the 300 – 500 mL water sample, using 0.22 µm or 0.45 µm pore size, 25 mm membrane filter. If the membrane filter size is too big, excise the membrane filter to 25 mm diameter after filtration. 3. Insert the membrane filter into the Soil Beads Tube by rolling the membrane, with the side containing trapped sample facing inward of the tube. ***Note:*** *If sample is not process immediately after Step 3, the* ***Soil Bead Tube*** *with the membrane filter can be stored at -20 °C. To continue the DNA extraction, thaw the sample to room temperature before start.*

Lysis	4. Add **800 µL SL1 Buffer** and **60 µL SL2 Buffer** into the **Soil Bead Tube**. 5. Homogenize the sample with max speed for 10 minutes. See Mechanical Disruption of Sample, page 4 for more information. 6. Short spin for 5 seconds to bring down the mixture and incubate at 65 ºC (water bath) for 10 minutes. 7. Centrifuge at 13,000 x *g* for 1 minute. 8. Remove the membrane filter from the **Soil Bead Tube** with forceps. 9. Transfer **600 µL supernatant** into a new 1.5 mL microcentrifuge tube. 10. Add **150 µL SL3 Buffer.** 11. Vortex to mix and incubate for 3 minutes at room temperature. 12. Centrifuge at maximum speed (≥ 13,000 x *g*) for 5 minutes.
Binding	13. Transfer the clear supernatant (~750 µL) to a new 2 mL microcentrifuge tube. 14. Add **equal volume of SBD Buffer (~750 µL)** and mix by inverting the tube 4 – 6 times. 15. Short spin to bring down the lysate. 16. Place a **PrimeWay Soil Column** into a new **Collection Tube**. 17. Transfer **up to 750 µL lysate** into the **PrimeWay Soil Column** and centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**.

Binding	18. Repeat Step 17 until all the sample has been transferred to the **PrimeWay Soil Column**.
Washing	19. Add **500 µL Wash Buffer S1** to the **PrimeWay Soil Column** and incubate for 1 minute. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 20. Add **650 µL Wash Buffer S2** to the **PrimeWay Soil Column**. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 21. Repeat Step 20.
Drying	22. Centrifuge the column at 10,000 x *g* for 1 minute to dry the column membrane.
Elution	23. Transfer the **PrimeWay Soil Column** to a new 1.5 mL microcentrifuge tube. 24. Add **50 µL preheated Elution Buffer (65 °C)** to the center of the **PrimeWay Soil Column** membrane. Incubate at room temperature for 3 minutes. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA. 25. Store the eluted DNA at 2 – 8 °C or -20 °C for long-term storage.

Support Protocol - DNA Clean-up

Preparation	I. Preheat the **Elution buffer** at 65 °C.
Binding	1. Transfer the DNA sample to a new 1.5 mL microcentrifuge tube. If the DNA volume is less than 300 µL, adjust the volume to 300 µL with **Elution Buffer**. 2. Add **300 µL SBD Buffer** and mix by inverting the tube 4 – 6 times. 3. Short spin to bring down the mixture. 4. Place a **PrimeWay Soil Column** into a new **Collection Tube**. 5. Transfer **600 µL sample mixture** into the **PrimeWay Soil Column** and centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 6. Add **500 µL SBD Buffer** to the **PrimeWay Soil Column**. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**.

Washing	7. Add **500 µL Wash Buffer S1** to the **PrimeWay Soil Column** and incubate for 1 minute. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 8. Add **650 µL Wash Buffer S2** to the **PrimeWay Soil Column**. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the column back into the **Collection Tube**. 9. Repeat Step 8.
Drying	10. Centrifuge the column at 10,000 x *g* for 1 minute to dry the column membrane.
Elution	11. Transfer the **PrimeWay Soil Column** to a new 1.5 mL microcentrifuge tube. 12. Add **50 µL preheated Elution Buffer (65 °C)** to the center of the **PrimeWay Soil Column** membrane. Incubate at room temperature for 3 minutes. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA. 13. [Optional] For maximum recovery, repeat Step 12 with a new 1.5 mL microcentrifuge tube. 14. 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	Isopropanol or/and ethanol is not added into the respective Wash Buffer	Ensure isopropanol and ethanol is added into Wash Buffer S1 & Wash Buffer S2 respectively. Mix well and repeat DNA extraction.
	Inappropriate binding condition	Ensure the volume of SBD Buffer is equal volume with the supernatant collected in Binding Step.
	Low nucleic acid content in the sample	Increase the sample input amount and/ or perform multiple DNA extraction.
		Repeat the DNA extraction without using SIR Buffer.
Colour eluent	Presence of soil contaminants	Proceed with DNA Clean-up protocol. Expected DNA recovery is 80 – 86 %.

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 (Adjustable speed)	For sample homogenization purposes