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PrimeWay Plasmid II DNA Extraction Kit (KIT-9041)

Molecular Biology Kits

PrimeWay Plasmid II DNA Extraction Kit

Product No: KIT-9041

The PrimeWay Plasmid II DNA Extraction Kit is designed for fast and efficient purification of high-quality plasmid DNA from bacterial cultures. It utilizes the alkaline lysis method combined with a silica spin column to isolate plasmid DNA up to 15 kb in size within just 25 minutes. The kit includes RNase A to eliminate RNA contamination during the process, ensuring clean and reliable results. The purified DNA is suitable for various downstream applications such as PCR, DNA sequencing, cloning, in vitro transcription, restriction digestion, and DNA labelling.

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

Kit Contents

No	Product	KIT-9041-10 10 preps	KIT-9041-50 50 preps	KIT-9041-250 250 preps
1	pD1 Buffer	3 mL	15 mL	70 mL
2	pD2 Buffer	3 mL	15 mL	75 mL
3	pD3 Buffer	4 mL	20 mL	100 mL
4	Wash Buffer A1	3.2 mL	16 mL	80 mL
5	Wash Buffer A2	1.5 mL	8 mL	40 mL
6	Elution Buffer	1.2 mL	5.5 mL	28 mL
7	RNase A	10 mg/mL	10 mg/mL	100 mg/mL
8	PrimeWay Plasmid II Column	10 pcs	50 pcs	5 x 50 pcs
9	Collection Tube	10 pcs	50 pcs	5 x 50 pcs

Storage

This kit should be stored at room temperature (21-25 °C). RNase A can be stored at room temperature for up to 2 years. For longer storage, store RNase A at −20 °C. After adding RNase A into pD1 Buffer, store at 4 °C.

Product Specification

	KIT-9041
Binding capacity	60 µg
Yield	Up to 40 µg
Sample	1–7 mL bacterial culture
Plasmid size	Up to 15 kb
Elution	50-100 µL
Duration	< 25 minutes

Materials Supplied by User

- ✓ Ethanol (96–100%)
- ✓ Centrifuge, at speed of 16,000 × *g*
- ✓ Vortex mixer
- ✓ 1.5 mL microcentrifuge tubes

Precautions for User

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

Before Start

- ✓ Prepare **pD1 Buffer containing RNase A** as below:
  i) Briefly centrifuge the RNase A tube. Add 100 µL of pD1 Buffer to the RNase A.
  Note: *Pipette gently to avoid splashing or spillage*
  ii) Vortex for 5 seconds or until pellet dissolves (if present) and transfer the entire solution back into the pD1 Buffer bottle.
  iii) Mix thoroughly and store the pD1 Buffer containing RNase A at 4 °C.
- ✓ Add ethanol (96–100%) to **Wash Buffer A1** and **Wash Buffer A2** as below and mix well:

P/No	Wash Buffer A1	Wash Buffer A2
KIT-9041-10	1.2 mL	6 mL
KIT-9041-50	6 mL	32 mL
KIT-9041-250	30 mL	160 mL

- ✓ Make sure no precipitation is observed in **pD2 Buffer**. Dissolve the precipitate by warming the pD2 Buffer in water bath at 37 °C.

Protocol – Plasmid II DNA Extraction

Sample	1. Pellet 1–7 mL of bacterial culture by centrifuging at 16,000 x *g* for 1 minute at room temperature. 2. Discard the supernatant completely.
Lysis	3. Resuspend the cell pellet in **250 µL of pD1 Buffer** by vortexing or pipetting up and down until no clumps are observed. ***Note**: Ensure that RNase A has been added to the pD1 Buffer.* 4. Transfer the cell suspension to a new 1.5 mL microcentrifuge tube. 5. Add **250 µL** of **pD2 Buffer** and mix gently by inverting the tube 10 times. 6. Incubate the lysate at room temperature for 3 minutes until the it becomes clear. ***Note**: Do not incubate for more than 5 minutes.* 7. Add **350 µL** of **pD3 Buffer** to neutralize the lysate. Mix immediately by inverting the tube 10 times. ***Note**: Do not vortex to avoid shearing genomic DNA.* 8. Centrifuge at maximum speed (~16,000 × *g*) for 10 minutes to pellet the cell debris.
Binding	9. Place the **PrimeWay Plasmid II Column** into a collection tube. 10. Transfer the clear supernatant into the **PrimeWay Plasmid II Column**. ***Note**: Do not transfer any white pellet into the column.* 11. Centrifuge at 16,000 × *g* for 30 seconds. Discard the flow-through and place the column back into the collection tube.

Washing	12. Add **400 µL Wash Buffer A1** into the **PrimeWay Plasmid II Column**. *Important: Ensure ethanol has been added to the buffer before first use.* 13. Centrifuge at 16,000 × *g* for 30 seconds and discard the flow-through. Place the column back to the Collection tube. 14. Add **700 µL Wash Buffer A2** into the **PrimeWay Plasmid II Column**. *Important: Ensure ethanol has been added to the buffer before first use* 15. Centrifuge at 16,000 × g for 30 seconds and discard the flow-through. Place the column back into the collection tube.
Drying	16. Centrifuge the column at maximum speed (~16,000 × *g*) for 3 minutes to remove residual Wash Buffer. ***Note**: Make sure the residual liquid is completely removed, as it can inhibit downstream applications.*
Elution	17. Place the **PrimeWay Plasmid II Column** into a new 1.5 mL microcentrifuge tube. 18. Add **50–100 µL Elution Buffer** to the centre of the **PrimeWay Plasmid II Column** membrane. 19. Incubate at room temperature for 2 minutes and centrifuge at maximum speed (~16,000 × *g*) for 2 minutes to elute the plasmid DNA. 20. Store the extracted plasmid DNA at -20 °C.

Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low plasmid yield	Poor lysis of bacterial cells	Ensure the cell pellet is completely resuspended before lysis. No cell clumps should be visible.
	Insufficient number of bacterial cells	Grow the bacterial cells longer at 37 °C under shaking but not more than 18 hours.
	Bacterial culture is too old	Do not overgrow the bacterial cells for more than 18 hours at 37 °C with shaking.
	DNA Elution	Ensure that the Elution Buffer is added to the centre of the column membrane and is completely absorbed by the column matrix.
	Wash Buffer was not prepared accordingly	Ensure ethanol has been added to Wash Buffer A1 and Wash Buffer A2 before use.
	Large plasmid size	Use Elution buffer preheated to 70 ˚C to improve recovery.
Presence of RNA contamination	Absence/ Insufficient volume of RNase A	Ensure that RNase A is added to the pD1 Buffer and thoroughly mixed.
Problem in the downstream processes	Ethanol contamination	Increase centrifugation time by an additional 3 minutes to ensure the Wash Buffer is completely removed.
Genomic DNA contamination	Vigorous lysis or prolonged incubation	• During the lysis step, gently invert the solution to mix, do not vortex. • Do not incubate the lysis mixture for more than 5 minutes.

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