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PrimeWay Plasmid DNA Extraction Kit (KIT-9040-50)

PrimeWay Plasmid DNA Extraction Kit

Product No: KIT-9040-50

The PrimeWay Plasmid DNA Extraction Kit is a rapid and reliable kit used to purify high quality plasmid DNA. It uses the alkaline lysis method to purify plasmid DNA from bacteria. This kit also comes with RNase A to remove RNA during extraction. It uses a silica-based spin column method and is suitable for extracting plasmid DNA up to 15 kb within 25 minutes. The purified plasmid is suitable for downstream application such as DNA sequencing, PCR, in vitro transcription, restriction mapping, cloning and DNA labeling applications.

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

Kit Contents

No	Product	KIT-9040-50	Storage
1	pD1 Buffer	15 mL	Room temperature (21 °C – 25 °C)
2	pD2 Buffer	15 mL	
3	pD3 Buffer	20 mL	
4	Wash Buffer A1	16 mL	
5	Wash Buffer A2	8 mL	
6	Elution Buffer	5.5 mL	
7	RNase A	1.5 mg	
8	PrimeWay Plasmid Column	50 pcs	
9	Collection Tube	50 pcs	

Product Specification

	KIT-9040-50
Binding capacity	60 µg
Yield	Up to 40 µg
Sample	1 – 5 mL bacterial culture
Plasmid size	Up to 15 kb
Elution	50 - 100 µL
Duration	< 25 minutes

Materials Supplied by Users

- ✓ Ethanol (96 – 100%)
- ✓ Centrifuge, at speed of 18,000 × *g*
- ✓ Vortex mixer
- ✓ 1.5 mL microcentrifuge tubes

Precautions for Users

- ✓ Some buffer of this kit contains irritants. Handle with care and avoid contact with skin. In case of contact, wash skin with plenty of water and seek medical attention.
- ✓ Always wear a lab coat, disposable gloves and surgical mask.

Protocol – Plasmid DNA Extraction

Preparation	I. Add **500 µL** of **pD1 Buffer** to RNase A and vortex to dissolve. Short spin to bring down the volume and transfer the RNase A solution to pD1 Buffer. Mix well and store **pD1 Buffer** at 4 °C II. Add **6 mL ethanol (96 – 100%)** into **Wash Buffer A1**. Mix well before use. III. Add **32 mL ethanol (96 – 100%)** into **Wash Buffer A2**. Mix well before use. IV. Make sure no precipitation observed in **pD2 Buffer**. Dissolve the precipitate by warming the pD2 Buffer in water bath at 37 °C.
Sample	1. Pellet the cells by centrifuging it at 11,000 x *g* for 1 minute. 2. Discard the supernatant.
Lysis	3. Resuspend the cell pellet with **250 µL pD1 Buffer** by vortexing or pipetting up and down until all cell pellet dissolved. ***Note**: Make sure that RNase A has been added into the pD1 Buffer.* 4. Transfer the cell suspension to a new 1.5 mL microcentrifuge tube. 5. Add **250 µL pD2 Buffer**, mix by gently inverting the tube for 5 – 10 times. 6. Incubate the lysate mixture at room temperature for 2 – 5 minutes until the lysate become clear. ***Note**: Do not vortex to avoid shearing of genomic DNA* *Do not incubate more than 5 minutes.* 7. Add **350 µL pD3 Buffer** to neutralize the lysate. Mix immediately by inverting the tube 5 – 10 times. 8. Centrifuge at maximum speed (~ 18,000 × *g*) for 10 minutes to pellet the cell debris.

Binding	9. Place the **PrimeWay Plasmid Column** into a Collection tube 10. Transfer the clear supernatant into the **PrimeWay Plasmid Column**. ***Note**: Do not transfer any white pellet into the column* 11. Centrifuge at 11,000 × *g* for 30 seconds. Discard the flow-through and place the column back to the Collection tube.
Washing	12. Add **400 µL Wash Buffer A1** into the **PrimeWay Plasmid Column**. *Important!! Make sure ethanol is added to the buffer prior to first use.* 13. Centrifuge at 11,000 × *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 Column**. *Important!! Make sure ethanol is added to the buffer prior to first use.* 15. Centrifuge at 11,000 × *g* for 30 seconds and discard the flow-through. Place the column back to the Collection tube.
Drying	16. Centrifuge the column at maximum speed (~ 18,000 × *g*) for 3 minutes to remove Wash Buffer residual. ***Note**: Make sure the residual liquid is completely removed as it can inhibit downstream application*.
Elution	17. Place the **PrimeWay Plasmid Column** into a new 1.5 mL microcentrifuge tube. 18. Add **50 – 100 µL Elution Buffer** to the center of the **PrimeWay Plasmid Column** membrane. 19. Incubate at room temperature for 1 minute and centrifuge at maximum speed (~ 18,000 × *g*) for 1 minute 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 bacteria cells	Ensure the cell pellet is completely resuspended before lysis. No cell clumps should be visible.
	Insufficient of bacterial cells	Grow the bacteria cells longer at 37°C under shaking but not more than 16 hours
	Bacteria culture is too old	Do not overgrown the bacterial cells for more than 16 hours at 37 °C under shaking.
	DNA Elution	Ensure that the Elution Buffer is added to the center of column membrane and 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 accordingly before use.
Presence of RNA contamination	Absence/ Insufficient volume of RNase A	Ensure that all the dissolved RNase A is added into pD1 Buffer.
Problem in the downstream processes	Ethanol contamination	Increase centrifugation time with additional 3 minutes to ensure the Wash Buffer is completely removed.
Genomic DNA contamination	Vigorous lysis/ long lysis incubation time	• During lysis step, gently invert the solution to mix, do not vortex. • Do not incubate the lysis mixture for more than 5 minutes

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