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PrimeWay Plasmid DNA Extraction Kit (KIT-9040)

Molecular Biology Kits

PrimeWay Plasmid DNA Extraction Kit

Product No: KIT-9040

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 labelling applications.

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

Kit Contents

No	Product	KIT-9040-10 10 preps	KIT-9040-50 50 preps	KIT-9040-250 250 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	5 µL	20 µL	80 µL
8	PrimeWay Plasmid 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 2 years. For longer storage, store RNase A at −20 °C. After adding in RNase A into pD1 Buffer, store at 4 °C.

Product Specification

	KIT-9040
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 User

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

Precautions for User

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

Before Start

- ✓ Prepare **pD1 Buffer containing RNase A** as below:
  i) Short spin RNase A tube. Add 100 µL of pD1 Buffer into RNase A.
  ii) Vortex to mix for 5 seconds or until pellet dissolve (if any) and transfer all solution back to pD1 Buffer.
  iii) Mix thoroughly and store 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-9040-10	1.2 mL	6 mL
KIT-9040-50	6 mL	32 mL
KIT-9040-250	30 mL	160 mL

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

Protocol – Plasmid DNA Extraction

Sample	1. Pellet 1 – 5 mL bacterial culture by centrifuging it at 11,000 x *g* for 1 minute. 2. Discard the supernatant completely.
Lysis	3. Resuspend the cell pellet with **250 µL pD1 Buffer** by vortexing or pipetting up and down until no clump observed. ***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 gently by 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. ***Note**: Do not vortex to avoid shearing of genomic DNA.* 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 centre 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 18 hours.
	Bacteria culture is too old	Do not overgrown the bacterial cells for more than 18 hours at 37 °C under shaking.
	DNA Elution	Ensure that the Elution Buffer is added to the centre 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 RNase A is added into pD1 Buffer and well-mixed.
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.

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