

PrimePrep™ Plasmid DNA Extraction Kit

Introduction

PrimePrep™ Plasmid DNA Extraction Kit offer simple, rapid and cost-effective method for isolating plasmid DNA from bacterial cells. This kit is designed for the preparation of up to 20 µg of high-purity plasmid DNA from 1 ~ 5 ml overnight *E. coli* culture in LB medium.

Plasmid DNA purified with mini kits is immediately ready for use. Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted in a small volume of Elution Buffer.

Kit Components

Reagents \ Cat. No.	K-1000 (50 prep.)	K-1002 (200 prep.)
Spin column	50 ea	50 ea x 4
Buffer PR	20 ml	55 ml
Buffer PL	20 ml	55 ml
Buffer PN	20 ml	75 ml
Buffer PO	20 ml	70 ml (35 ml x 2)
Buffer PW	10 ml	30 ml (15 ml x 2)
Buffer PE	10 ml	20 ml
RNase A Solution (10 mg/ml)	200 µl	550 µl

Before you begin

- ▶ Add RNase A Solution to Buffer PR, mix, and store at 4°C.
- ▶ Add ethanol to Buffer PO before use.
→ Add 12 ml (K-1002: 21 ml) of absolute ethanol before use.
- ▶ Add ethanol to Buffer PW before use.
→ Add 40 ml (K-1002: 60 ml) of absolute ethanol before use.
- ▶ Check Buffer PL and PN before use for salt precipitation.

Note: Redissolve any precipitation by warming to 50°C.

Do not shake Buffer PL vigorously.

Experimental Protocol

- Growth of bacterial culture in tubes or flasks.

* Harvest the bacterial cells by centrifugation at 8,000 rpm in a conventional, table-top microcentrifuge for 3 min at room temperature.

1. Resuspend pelleted bacterial cells in 250 μl of Buffer PR and transfer to a microcentrifuge tube.

The bacterial cell should be resuspended completely by vortexing and pipetting.

2. Add 250 μl of Buffer PL and gently mix by inverting the tube 4 ~ 6 times.

Incubate at room temperature for less than 5 min. **Do not vortexing.** Vortexing may cause shearing of genomic DNA.

3. Add 350 μl of Buffer PN and mix immediately and thoroughly by gently

inverting the tube 4 ~ 6 times. **Do not vortexing.** To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer PN.

4. Centrifuge for 10 min at maximum speed in a table-top-microcentrifuge.

A compact white pellet will form.

5. Transfer the supernatant to the Spin column by decanting or pipetting.

Avoid the white precipitation co-transferring into the Spin column.

6. Centrifuge for 30 ~ 60 sec. Pour out the filtrate and re-inserting the Spin column to the collection tube.

7. (Optional step) Add 500 μl Buffer PO and centrifuge for 30 sec.

This step is only required when using *endA*⁺ or other bacteria strains with high nuclease activity or carbohydrate content.

8. Add 700 μl Buffer PW and centrifuge for 30 sec.

Pour out the filtrate and re-inserting the Spin column to the collection tube.

9. Centrifuge for an additional 1 ~ 2 min to remove residual wash buffer.

Residual ethanol of washing buffer may inhibit subsequent enzymatic reactions.

10. Transfer the Spin column into a clean 1.5 ml microcentrifuge tube (Not provided).

11. Add 50 μl of Buffer PE (10mM Tris-HCl, pH 8.5) or deionized distilled water, let stand for 1 min and centrifuge for 1 min.