# *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 20 ㎍ 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

| Cat. No. / Reagents | K-1000 (50 prep.) |
|---|---|
| Spin column | 50 ea |
| Buffer PR | 20 ml |
| Buffer PL | 20 ml |
| Buffer PN | 20 ml |
| Buffer PO | 20 ml |
| Buffer PW | 10 ml |
| Buffer PE | 10 ml |
| RNase A Solution (10 mg/ml) | 200 ㎕ |

## Before you begin

- ▶ **Add RNase A Solution to Buffer PR, mix, and store at 4℃.**
- ▶ **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℃.**
**Do not shake Buffer PL vigorously.**

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## 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 ㎕ of Buffer PR and transfer to a microcentrifuge tube.**
   The bacterial cell should be resuspended completely by vortexing and pipetting.
2. **Add 250 ㎕ 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 case shearing of genomic DNA.
3. **Add 350 ㎕ 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 ㎕ 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 ㎕ 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 ㎕ of Buffer PE (10mM Tris-HCl, pH 8.5) or deionized distilled water, let stand for 1 min and centrifuge for 1 min.**