Kit Contents

Plasmid Miniprep Kits	50 preps	200 preps
Cat. No.	A510-50	A510
Cell Resuspension Buffer (Sol Ⅰ)	13 ml	50 ml
Cell Lysis Buffer (Sol Ⅱ)	13 ml	50 ml
Neutralization Buffer* (Sol Ⅲ)	18 ml	70 ml
Denaturation Buffer*	25 ml	100 ml
Wash Buffer** (Sol Ⅳ, concentrate)	10 ml	40 ml
Elution Buffer	5 ml	20 ml
RNase A Buffer	130 μl	500 μl
Collection tubes	50	200
Plasmid Miniprep Columns	50	200
Protocol	1	1

*Neutralization Buffer, Denaturation Buffer contain chaotropic salts and wear gloves when using these kits.
**Add absolute ethanol before use (see Working Solution part and bottle labels)

Storage and Stability

Store at room temperature and used as described in this manual.
(exception: after adding RNase A to Cell Resuspension Buffer , store at 2-8℃)
Guaranteed for 12 months from the date of purchase.

Working Solution

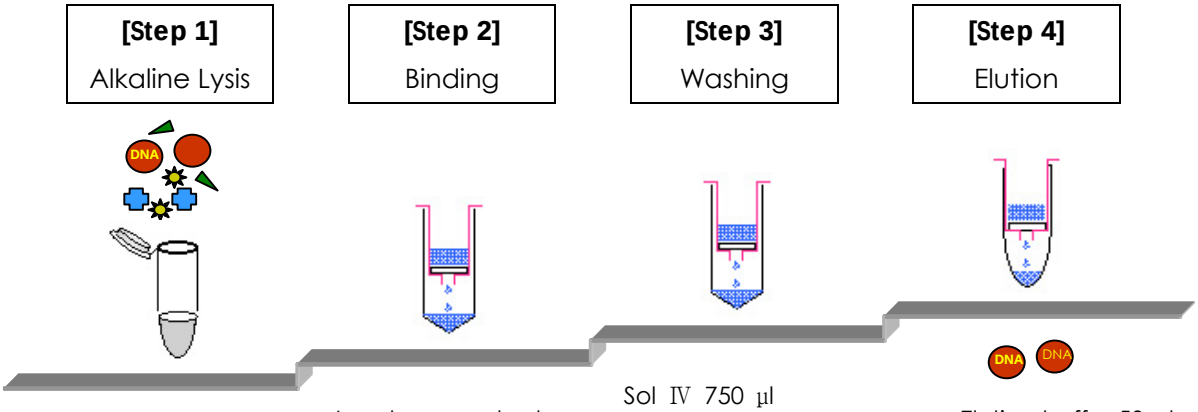
	50 preps	200 preps	200 preps (A560)
Wash Buffer (concentrate)	10 ml	40 ml	20 ml X 2
Absolute ethanol	Add 40 ml	Add 160 ml	Add 80 ml

Plasmid Miniprep Kit

I. Introduction

Plasmid Miniprep system provides fast and simple method for purifying plasmid DNA from bacterial cells. This kit uses the optimized alkaline lysis method and spin columns with a glass fiber membrane bind up to 20ug of plasmid DNA in the presence of chaotropic salts. Miniprep can be processed within 30 minutes and requires no phenol extraction and ethanol precipitations. Purified plasmid DNA is ready for restriction enzyme digestion, ligation, transformation, sequencing reactions, and library screening.

II. Simple procedures



III. Note

- Additional material: Absolute ethanol, Microcentrifuge tube for elution (1.5 ml), Tabletop microcentrifuge
- Maximum speed of all steps is more than 13,000rpm.

1. Add provided RNase A Buffer to Sol Ⅰ(Cell Resuspension Buffer) and store at 2-8℃. The RNase A buffer should retain activity for up to 6 months. After longer periods of storage, RNase A may not effectively eliminate RNA. In this case, add more RNase A Buffer.
2. If precipitate have formed in Sol Ⅱ(Cell Lysis Buffer), dissolve by warming at 37℃. Close the bottle immediately after use to avoid acidification of Sol Ⅱ from CO_2 in the air.
3. If Sol Ⅲ(Neutralization Buffer) is precipitated, dissolve by warming at 37℃.
4. Add the indicated volume (40ml/160ml) of absolute ethanol to Sol Ⅳ(Wash Buffer) (see page 3, Working Solution).
5. RNase A Buffer can be stored at room temperature. For longer storage, it should be kept at 2-8°C.
6. Neutralization Buffer and Denaturation Buffer contain chaotropic salt. Wear gloves and goggles when handling.
7. If water is used for elution, make sure that its pH is between 7.0 and 8.5.
   Store DNA at -20℃ as DNA may degrade in the absence of a buffer agent. DNA can also be eluted in TE (10mM Tris-HCl, 1mM EDTA, pH 8.0), but EDTA may inhibit subsequent enzymatic reaction.

IV. Protocol

1. Harvest bacterial cells

Transfer 1~10 ml bacterial culture to a microcentrifuge tube. Centrifuge and discard the supernatant.

✓ Processing more cells can overload the cartridge system and result in lower plasmid DNA yield. Harvest 1~5 ml (high copy number plasmid) or 10 ml (low copy number plasmid) of bacterial culture.

2. Resuspension

Add 250 μl of Cell Resuspension Buffer (Sol Ⅰ, RNase A added). Resuspend the cell pellet by vortexing or pipetting.

✓ No cell clumps should be visible after resuspension of the pellet. Lysis efficiency highly depends on complete resuspension.

3. Lysis

Add 250 μl of Cell Lysis Buffer (Sol Ⅱ) and gently invert the tube 4~6 times.

✓ Total lysis time should not exceed 5 min. Do not vortex, as this will result in shearing of genomic DNA. The solution should become slightly clear.

4. Neutralization

Add 350 μl of Neutralization Buffer (Sol Ⅲ) and invert the tube immediately 4~6 times.

✓ Do not vortex. The solution should become cloudy and form white flocculants.

5. Pellet the Cell Debris

Centrifuge at maximum speed (>13,000 rpm) for 10 min.

✓ Compact white debris form. The supernatant contains the plasmid DNA.

6. Binding DNA

Place a Plasmid Miniprep Column in a 2 ml Collection tube.

Transfer the supernatant from step 5 to the prepared Plasmid Miniprep Column by pipetting.

Centrifuge at maximum speed for 1 min. Discard the flow-through and place the column back into the collection tube.

7. [Optional Washing]

[Optional] Add 500 μl Denaturation Buffer and centrifuge at maximum speed for 1 min. Discard the flow-through and place the column back into the collection tube.

✓ If used *E.coli* strains have high nuclease activity (*e.g.*, endA+ strains, see page 9, table 2), perform this step. They degrade plasmids and can be problematic for several applications. If *E.coli* strain is endA-, proceed with step 8.

✓ If plasmid DNA is large size (>10kb) or low copy number, this step is recommended.

8. Washing

Add 750 μl Wash Buffer (Sol Ⅳ, ethanol added) and centrifuge at maximum speed for 1 min. Discard the flow-through and place the column back into the collection tube.

✓ If salt or nuclease is remained in this step, add Wash Buffer, let stand for 2 min, and then centrifuge for 1min.

9. Drying

Centrifuge for an additional 1 min to remove residual Wash Buffer.

✓ Residual ethanol from Wash Buffer may inhibit subsequent enzymatic reaction.

10. Elution

Place a Plasmid Miniprep Column in a 1.5 ml microcentrifuge tube. Add 50~100 μl of Elution Buffer to the center of each Plasmid Miniprep Column. Wait for 1 min and centrifuge at maximum speed for 1 min.

✓ Elution can be done with sterile deionized water or TE buffer. However TE buffer contained EDTA may interfere with subsequent reactions

✓ In the case of large plasmid (>10kb), use pre-warmed (70℃) Elution buffer.

✓ Store the purified plasmid DNA at -20℃ or below.

V. Troubleshooting

1. Low or no yield of Plasmid DNA

The yield and quality of prepared plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic, and type of culture medium.

(1) Ethanol not added to Wash Buffer.

Prepare the Wash Buffer as instructed before beginning the procedure. (see page 3, Working Solution)

(2) Incomplete lysis of bacterial cells.

✓ Poor resuspension of bacterial cell pellet.

The cell pellet must be thoroughly resuspened prior to cell lysis. No clumps should be visible after resuspension.

✓ Too many bacterial cells were used.

The use of excessive culture volumes may lead to a biomass value too high for complete lysis. If too much culture volume is used, alkaline lysis will be inefficient, the plasmid miniprep membrane will be overloaded, and the performance of the system will be unsatisfactory.

✓ Cell Lysis Buffer is precipitated.

SDS may precipitate Cell Lysis Buffer. If precipitated, incubate at 30~40°C and mix.

(3) Cell inoculated in medium containing insufficient amount of antibiotics.

Antibiotic selection should be applied at all stages of growth. Non-transformed cells replicate much faster than plasmid-containing cells in the absence of selective pressure, and can quickly take over the culture. The stability of the selective agent should also be taken into account.

Table 1. Concentrations of commonly used antibiotics

	Stock solution		Working concentration
	Concentration	Storage	
Ampicillin	50 mg/ml in H_2O	-20℃	100 μg/ml
Chloramphenicol	34 mg/ml in ethanol	-20℃	170 μg/ml
Kanamycin	10 mg/ml in H_2O	-20℃	50 μg/ml
Streptomycin	10 mg/ml in H_2O	-20℃	50 μg/ml
Tetracycline HCl	5 mg/ml in ethanol	-20℃	50 μg/ml

(4) Low copy number plasmid used.

Increase culture volume up to 10ml or use high copy number plasmid.

Plasmids vary widely in their copy number per cell, depending on their origin of replication (e.g., pMB1, ColE1, or pSC101) that determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert.

(5) Bacterial cultures too old or overgrowth

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid. A single colony should be inoculated into media containing the appropriate selective agent, and grown with vigorous shaking for 12-16 hours. Growth for more than 16 hours is not recommended since cells begin to lyses and plasmid yields may be reduced.

(6) Elution buffer incorrect or incorrectly dispensed onto membrane

Add elution buffer to the center of plasmid miniprep column. Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH7.0 and 8.5. When using water for elution, make sure that the pH value is within this range.

(7) Nuclease rich host strain used.

If using nuclease rich strain (EndA+ *E.Coli* strains) like HB101 or JM series, perform the optional denaturation washing step to remove endonuclease. (see page 6, step 7)

Table 2. EndA- and EndA+ strains of *E.Coli.*

EndA-		EndA+	
BJ5183	JM108	BL21 (DE3)	P2392
DH1	JM109	CJ236	PR series
DH20	MM294	HB101	Q358
DH21	SK1590	JM83	RR1
DH5aTM	SK1592	JM101	TB1
JM103	SK2267	JM110	TG1
JM105	SRB	LE392	Y10 series
JM106	XL-Blue	MC1061	BMH71018
JM107	XLO	NM series	ES1301

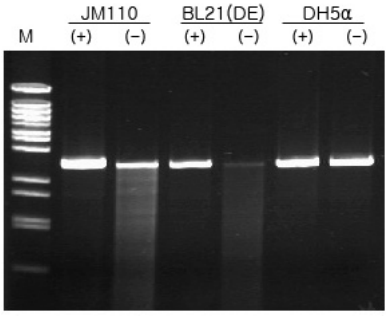


Figure 1. Agarose gel analysis of pBluescript II KS(+) vector after restriction enzyme digestion.
M: size marker
(+): Add Denaturation step
(–): No Denaturation step

2. Genomic DNA contamination

Do not vortexing or overmixing after addition of Cell Lysis Buffer to prevent shearing of genomic DNA.

3. Nicked Plasmid DNA

Too long lysis time after addition of Cell Lysis Buffer can cause nicking of plasmid DNA. Lysis time should not be over 5min.

VI. Experimental Data

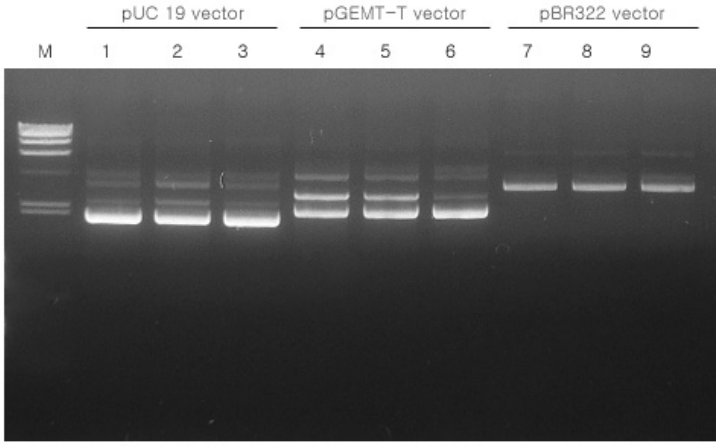


Figure 2. Analysis of plasmid DNA purified by Plasmid Miniprep Kit.
lane M: λ/*Hind*Ⅲ marker, lane 1,4,7: supplier A, lane 2,3,5,6,8,9: Plasmid Miniprep Kit

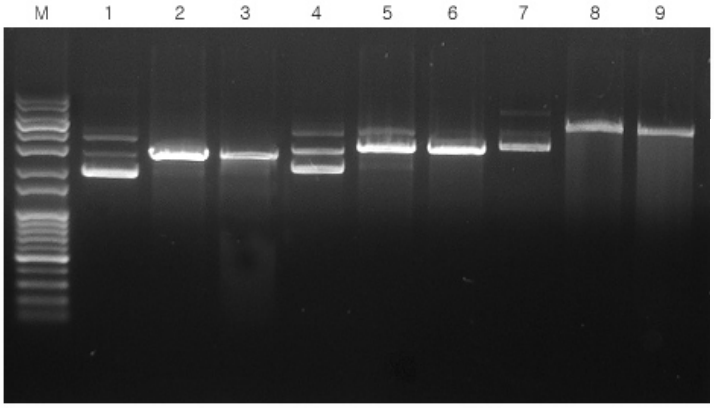
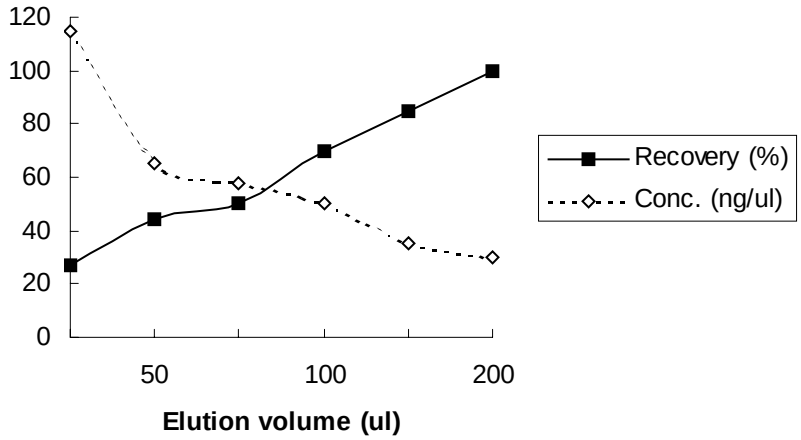


Figure 3. Analysis of plasmid DNA digested with restriction enzyme.
lane M: 1kb plus 100bp DNA ladder marker, lane 1: pUC19(uncut), lane 2: pUC19 (*EcoR*Ⅰ), lane 3: pUC19 (*Hind*Ⅲ), lane 4: pGEM-T (uncut), lane 5: pGEM-T (*EcoR*Ⅰ), lane 6: pGEM-T (*sal*Ⅰ), lane 7: pBR322 (uncut), lane 8: pBR322 (*EcoR*Ⅰ), lane 9: pBR322 (*Hind*Ⅲ)



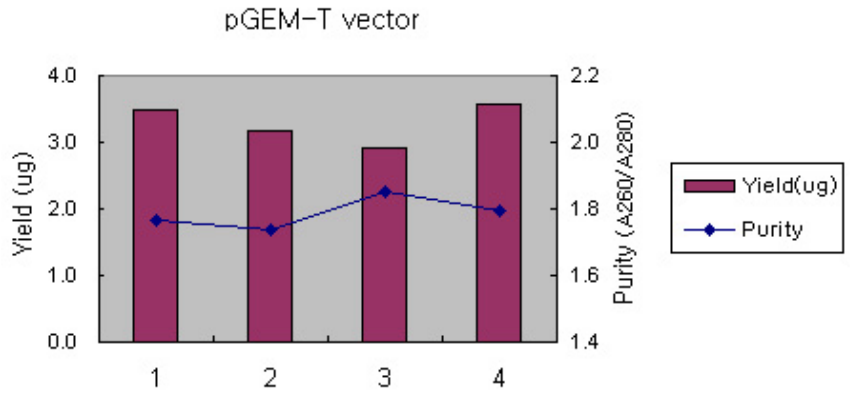


Figure 4. Yield and purity of plasmid DNA using Plasmid Miniprep Kit.
lane 1: supplier A, lane 2: supplier B, lane 3: supplier C, lane 4: Plasmid Miniprep Kit

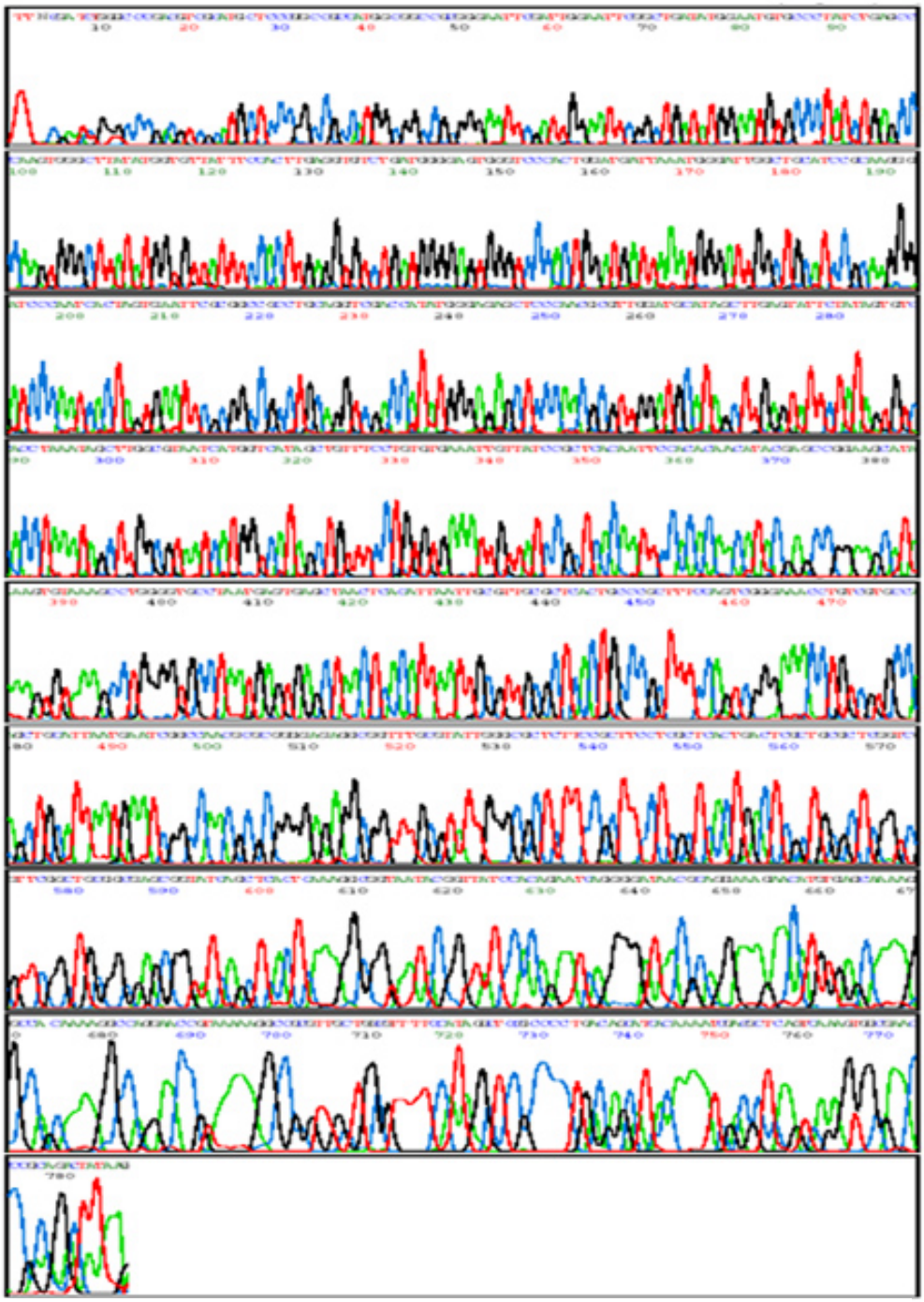


Figure 5. Electropherogram revealing >700 bases of sequence from pGEM-T vector using Plasmid Miniprep Kit.