Plasmid Miniprep Kit

Contents

Important Notes

Plasmid Copy Numbers: The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 2 to 3 times.

Plasmid	Origin	Copy Numbers	Expected Yield (µg per 1 mL)
pSC101	pSC101	5	0.1-0.2
pACYC	P15A	10-12	0.4-0.6
pSuperCos	pMB1	10-20	0.4-1
pBR322	pMB1	15-20	0.6-1
pGEM®	Muted pMB1	300-400	6-7
pBluescript®	ColE1	300-500	6-8
pUC	Muted pMB1	500-700	8-12

Host Strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10, DH5a, and C600 yield high-quality plasmid DNA. endA+ strains such as JM101, JM109, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield. We recommend transform plasmid to a endA- strain if the yield is not satisfactory. For endA+ strains, we recommend use Buffer KB to remove the endonuclease before washing step.

Culture Medium: This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) to density of OD_{600} 2.0 to 3.0. If rich medium such as TB or 2xYT are used, make sure the cell density doesn't exceed 3.0 (OD_{600}). A high ratio of cell density over lysis buffer results in low DNA yield and purity. For over amount of cell numbers, either reduce the biomass or scale up the volumes of Buffer A1, B1 and N1.

Introduction

Key to the kit is our proprietary DNA binding system that allows the highly efficient binding of DNA to our matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with Elution buffer. The purified DNA is ready for downstream applications such as cloning/subcloning, RFLP, sequencing, and transfection of HEK293 cells. The yield from 1 mL culture is typical around 8 to 12 ug. The mini column has a DNA binding capacity of 40 ug.

Storage and Stability

Buffer A1 should be stored at 4°C once RNase A is added. All other materials can be stored at room temperature. The Guaranteed shelf life is 18 months from the date of purchase.

Kit Contents

Catalog Number	PD1211-01	PD1211-02
Preps	50	250
ezBind Columns	50	250
Buffer A1	15 mL	65 mL
Buffer B1	15 mL	65 mL
Buffer N1*	20 mL	90 mL
Buffer KB	15 mL	70 mL
DNA Wash Buffer**	12 mL	50 mL
Elution Buffer	10 mL	30 mL
RNase A	50 µL	210 µL

*Buffer N1 contains chaotropic salts, wear gloves and protective eyewear when handling.

**Add 48 mL (D1211-01) or 200 mL (D1211-02) 96-100% ethanol to DNA Wash Buffer before use.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important:

- **RNase A**: Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use.
- Add 48 mL (1211-01) or 200 mL (D1211-02) 96-100% ethanol to DNA Wash Buffer before use
- Buffer B1 precipitates below room temperature, it is critical to warm up the buffer at 37°C to dissolve the precipitates before use.
- Keep the cap tightly closed for buffer B1 after use.
- Ensure the availability of centrifuge capable of 12,000 rpm.
- Carry out all centrifugations at room temperature.
- Buffer N1 contains a chaotropic salt, wear gloves and protective eyewear when handling.

Materials supplied by users:

- 96- 100% ethanol.
- 1.5 mL microcentrifuge tubes
- High speed microcentrifuge.

EZgene ™ Plasmid Miniprep Protocol

1. **Inoculate 1-5 mL LB containing appropriate antibiotic with a fresh colony. Grow at 37°C for 14-16 h with vigorous shaking.**

2. **Harvest the bacterial culture by centrifugation for 1 minute at 12,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.**

   Note: This protocol is optimized for *E. coli* strain cultured in LB medium. When using TB or 2xYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers needs to be scaled up if over amount of cultures are being processed.

2. **Add 250 µL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).**

3. **Add 250 µL Buffer B1, mix gently by inverting 10 times (do not vortex) and incubate at room temperature for 5 minutes until the solution becomes clear.**

4. **Add 350 µL Buffer N1, mix completely by inverting the vial for 5 times.**
   Note: It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mixing is required to completely neutralize the solution.

5. **Centrifuge the lysate at 12,000 rpm for 10 minutes at room temperature.**

6. **Carefully transfer the clear lysate into a DNA column with a collection tube, avoid the precipitations, spin at 12,000 rpm for 30s, discard the flow-through and put the column back to the collection tube.**
   Note: If the lysate doesn't appear clean, centrifuge for 5 more minutes and then transfer the clear lysate to DNA column.

   Optional: Add **300 µL Buffer KB** and centrifuge at 12,000 rpm for 30s. Discard the flow-through and put the column back to the collection tube. Note: This step is NOT necessary if the plasmid is being purified from endA-strain such as DH5a and Top 10. It is recommended for endA+ strains such as HB101, JM101, JM110 and their derived strains.

7. **Add 600 µL DNA Wash Buffer into the column, and centrifuge at 12,000 rpm for 1 minute at room temperature. Discard the flow-through.**

9. **Reinsert the spin column into the collection tube and centrifuge for 2 minutes at 12,000 rpm.**

10. **Carefully transfer the column into a clean 1.5 ml tube and add 50 - 100 µL Elution Buffer into the column and let it stand for 1 minute. Elute the DNA by centrifugation at 12,000 rpm for 1 minute.**

    Note: The first elution yields approximately 70 % of the plasmid DNA.
    Optional: Add the eluted DNA back to the column and centrifuge at 12,000 rpm for 1 min to elute the DNA. The second elution yields another 10-20 % of the plasmid DNA.

11. **The DNA concentration can be calculated as follows,**

    DNA concentration = Absorbance 260 nm x 50 x dilution factor (µg/ml).

    Note: The DNA is ready for down stream applications such as cloning, sequencing, RFLP or transfection of 293 cells.

    Note: It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

    Note: Use less elution buffer if high DNA concentration is desired.

Trouble Shooting Guide

Low Yield	Poor Cell lysis.	• Resuspend pellet thoroughly by votexing and pipeting prior adding buffer B1. • Make fresh buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2N NaOH and 1%SDS).
Low Yield	Bacterial culture overgrown or not fresh.	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at -20°C if the culture is not purified the same day. Do not store culture at 4°C over night.
Low Yield	Low copy-number plasmid.	Increase culture volume (up to 10mL for Minipreps, 200mL for Midipreps, 400 mL for Maxipreps and 3L for Megapreps). Scale up the volume of buffers accordingly.
No DNA	Plasmid lost in Host *E.coli*	Prepare fresh culture.
Genomic DNA contamination	Over-time incubation after adding Buffer B1.	Do not vortex or mix aggressively after adding buffer B1. Do not incubate more than 5 minutes after adding solution B1.
RNA contamination	RNase A not added to Buffer A1.	Add RNase A to buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	Ethanol traces not completely removed from column.	Make sure that no ethanol residual remaining in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.

Removal Procedure

This protocol is designed to remove endotoxin after the plasmid DNA is purified (Buffers can be scaled up or down accordingly).

1. Add **0.2 volume** of **EndoClean buffer** to the plasmid sample in a 1.5 mL sterile tube. (For example, add 0.2 mL endoClean buffer to 1 mL plasmid sample).

2. Mix by vortexing the tube a few times and put on ice for about 10 minutes until the solution is clear without turbidity. (Rocking the sample in a cold room for 10 minutes is recommended if it is available).

3. Mix well again by inverting the tube a few times.

4. Incubate the tube at 55 °C water bath for about 5 minutes and the solution shall be turbid.

5. Centrifuge at top speed at **room temperature** for 5 minutes with the brake off.

6. Carefully transfer the upper clear layer solution to 1.5 mL tube. Split in two 1.5 mL tubes, 400- 450 uL each tube.

7. Precipitate plasmid DNA with 0.1 volume of 3 M NaAc (pH 5.2) and 2 volume of 100% ethanol.

Note: The EndoClean-treated plasmid DNA is ready transfection of endotoxin sensitive cell lines.