EZgene ™ Plasmid Miniprep II Protocol

Contents

Important Notes

Plamsid 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 2-3 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^{R}	Muted pMB1	300-400	6-7
pBluescript^{R}	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, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommend transform plasmid to an *endA*- strain if the yield is not satisfactory. For purifying plasmid DNA from endA+ strains, we recommend extra wash step by Buffer KB.

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 ezBind^{TM} matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or Elution Buffer. The purified DNA is ready for downstream applications such as cloning/subcloning, RFLP, sequencing, and transfection of HEK293 cells.

Storage and Stability

Buffer A1 should be stored at 4^{o}C once RNase A is added. All other materials can be stored at room temperature. The Guaranteed shelf life is 12 months from the date of purchase.

Kit Contents

Catalog Number	PD1213-01	PD1213-C
Preps	50	100
Mini Column II	50	100
Buffer A1	25 mL	60 mL
Buffer B1	25 mL	60 mL
Buffer N1*	30 mL	75 mL
Buffer KB*	15 mL	75 mL
DNA Wash Buffer**	12 mL	50 mL
Elution Buffer	15 mL	40 mL
RNase A	110µL	200µL

**Buffer N1 and Buffer KB contain chaotropic salts, wear gloves and protective eyewear when handling.
**Add 48 mL (D1213-02) or 200 mL (D1213C) 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 step.

Important:

- This kit is designed to purify up to 100 µg plasmid DNA from 6-12 mL culture (See protocol on Page 4). This kit can also be used to purify plasmid DNA from 1-5 mL culture just like regular miniprep (See protocol on Page 8).
- **RNase A**: Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use.
- Buffer B1 precipitates below room temperature, it is critical to dissolve at 37°C before use. Keep the cap tightly closed for buffer B1 after use.
- Carry out all centrifugations at room temperature.
- Buffer N1 and Buffer KB contain a chaotropic salt, wear gloves and protective eyewear when handling.

Materials supplied by users:

- 96- 100% ethanol.
- 1.5 mL, 2.0 microcentrifuge tubes.
- 15 mL conical tubes.
- High speed microcentrifuge.
- ddH_2O.

For processing 1-5 mL culture, please follow protocol on page 8.

1. Inoculate 5-12 mL LB containing appropriate antibiotic with a fresh colony. Grow at 37°C for 14-16 h with vigorous shaking.

2. Harvest bacterial culture by centrifugation for 1 minute at 12,000 rpm for 1 min. 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.

   Note: The culture can be centrifuged at 6,000 rpm in a 15 mL conical tube for 5 minutes if high speed centrifuge tubes are not available. Alternatively, the cultures can also be spin down in multiple 2.0 mL tubes.

3. Add 450 µL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).

4. Add 450 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. Do not incubate for more than 5 minutes.

   Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 50°C to dissolve precipitation before use

5. Add 550 µL Buffer N1, mix completely by inverting/ shaking the vial for 5-10 times. It is critical to mix the sample well. If the mixture still appears conglobated, brownish or viscous; more mixing is required to completely neutralize the solution.

6. Transfer the lysate to a 2 mL microtube and centrifuge at 12,000 rpm for 10 minutes at room temperature.

7. Carefully transfer 750 µL clear lysate into a DNA column with a collection tube, avoid the precipitations, spin at 12,000 rpm for 20 seconds, discard the flow-through and put the column back to the collection tube.
   Note: If the lysate doesn't appear clean, reverse the tube angle, centrifuge for 5 more minutes and then transfer the clear lysate to the DNA column.

8. Carefully transfer the remaining clear lysate to the column and centrifuge at 12,000 rpm for 30 seconds at room temperature. Discard the flow-through. Put the column back to the collection tube.

9. Optional: Add 300 µL Buffer KB into the spin column, centrifuge at 12,000 rpm for 1 minute. Remove the spin column from the tube and discard the flow-through.

   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.

10. Add 700 µL DNA Wash Buffer into the spin column, centrifuge at 12,000 rpm for 30 seconds at room temperature. Discard the flow-through.

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

    Note: It is critical to remove the residual ethanol for optimal DNA elution.

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

13. Carefully transfer the column into a clean 1.5 ml tube and add 100-150 µL Elution Buffer into the column. Let it stand for 1 minute. Elute the DNA by centrifugation at 12,000 rpm for 1 minute.
    Optional: The first elution normally yields 60-70% of the DNA. Add the eluted DNA back to the column for a 2nd elution yields another 20-30% of the DNA.

14. 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 20 mL 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 solution A1.	Add RNase A to buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze 75or 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.

This kit can also be used to process 1-5 mL colure just like regular miniprep. Follow protocol on Page 4 if processing 6-12 mL culture.

1. Harvest *1-5 mL* of fresh 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.
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. **Do not incubate for more than 5 minutes.**
4. Add 350 µL Buffer N1, mix completely by inverting/sharp hand shaking the vial for 5 times.
5. Centrifuge 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, reverse the tube angle, centrifuge for 5
   more minutes and then transfer the clear lysate to DNA column.
7. 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.
8. Add 600 µL DNA Wash Buffer *(Add ethanol to DNA wash buffer before use)* into the spin column, centrifuge at 12,000 rpm for 1 minute at room temperature. Remove the spin column from the tube and discard the flow-through. Optional: Repeat step "8".
9. Reinsert the spin column, with the lid open, into the collection tube and centrifuge for 1 minute at 12,000 rpm.
10. Carefully transfer the spin column into a clean 1.5 ml tube and add 60-100 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.
    Optional: For maximum yield and concentration, add the eluted DNA back to the column and spin at 12,000 rpm for 1 minute.