Plasmid Midiprep Kit II

Contents

Important Notes:

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 3 to 5 times. Please contact our customer service for further information and reference the table below for the commonly used plasmids,

Plasmid	Origin	High copy	Low copy
pACYC	P15A		10-12
pSC101	pSC101		5
pSuperCos	pMB1		10-20
pBR322	pMB1		15-20
pUC	Muted pMB1	500-700	
pGEM®	Muted pMB1	300-400	
pBluescript®	ColE1	300-500	

Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our ezBind™ matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or TE buffer. Unlike all other rivals, Biomiga's patented plasmid purification kit has no guanidine salt in the buffer, the purified DNA is guanidine/ion exchange resin residues free which enable the high performance of downstream applications such as transfection, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations.

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	D1412-01	D1412-02
Preps	10	25
Midi Columns	10	25
Buffer A1	60 mL	130 mL
Buffer B1	60 mL	130 mL
Buffer C1*	80 mL	170 mL
Elution Buffer	15 mL	30 mL
DNA Wash Buffer**	50 mL	2x 50 mL
RNase A	200 µL	420 µL

*Buffer C1 contains acetic acid, wear gloves and protective eyewear when handling.
**Add 200 mL 100% ethanol to each 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.
- 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 13,000 rpm.
- Carry out all centrifugations at room temperature.

Materials supplied by users:

- 100% ethanol
- Miniprep: 1.5 mL eppendorf tubes and centrifuge.
- Midi/maxiprep: high speed centrifuge, 30 mL high speed centrifuge tubes, 15 /50 ml tubes.

EZgene TM Plasmid Midiprep II Protocol

1. Inoculate 50-80 mL LB containing appropriate antibiotic with 100 µL fresh starter culture. Grow at 37oC for 14-16 h with vigorous shaking.
Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 ml LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 h with vigorous shaking (~250 rpm). Warning: Do not use more than 100 ml culture. Need to scale up buffers if processing more than 80 mL culture.

2. Harvest the bacterial by centrifugation at 5,000 g for 10 min at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.
Note: Complete removal of residue medium is critical for bacteria lysis in the next step.

3. Add 5 mL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting. Ensure that RNase A has been added into Buffer A1 before use.
Note: Complete resuspension is critical for optimal yield.

4. Add 5 mL Buffer B1, mix thoroughly by inverting 10 times with slightly shaking. Incubate for 5 min to obtain a slightly clear lysate. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.
Attention: Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37oC to dissolve before use.

5. Add 2.5 mL Buffer C1, mix completely by inverting 10 times sharp shaking for 2 times.

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.

6. Two options for clearing the lysates:

High speed centrifuge: Transfer the lysate to a high speed centrifuge tube and centrifuge at 13,000 rpm for 15 min at room temperature. Transfer the cleared lysate to a 50 ml conical tube. Add 3.0 mL Buffer C1 and mix well. Go to step 7.

ezFilter syringe: Incubate the lysate at room temperature for 10 min. Add 3.0 mL Buffer C1 and mix well. Pour the lysate into the barrel of the filter syringe. Hold the syringe for 30 seconds over a clean 50 ml conical tube. The white precipitates should float to the top. Gently insert the plunger to expel the cleared lysate to the tube, stop when feel resistance, some of the lysate may remain in the flocculent precipitate.
Note: To avoid clog of the syringe: Use less than 100 mL of overnight culture and mix the lysate well after adding Buffer C1.

7. Add 5 mL absolute ethanol (96-100%) to the cleared lysate. Mix well by sharp shaking for 5 times. Proceed with centrifugation protocol described below.

8. Pull out the plunger from the DNA column and set the column in a 50 mL conical tube. Add the lysate/ethanol mixture into a DNA column. Using the plunger, gently expel the lysate through the column to the conical tube.

9. Gently pull the plunger out, add 10 mL DNA Wash Buffer, and expel the Buffer out with the plunger.

10. Use the plastic wrench (Provided) to detach the end component from the midiprep column and insert it into a 1.5 ml eppendorf tube.

11. **Centrifuge** the column at 13,000- 15,000 rpm (Max speed) for 1 min. **Decant** the flow through and put the column back to the tube. Spin the column at max speed for 1 min. Transfer the column to a new eppendorf tube.

12. **Add 0.4 mL Elution Buffer to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 13,000 rpm for 1 min.**

13. Transfer the column to a new 1.5 mL tube and add **0.2 mL Elution Buffer** to the column for a second elution. The first elution yields 60-70% of the DNA while the second elution yields another 20-30% of the DNA bound to the column.

Note: The DNA is ready for downstream applications such as cloning, RFLP, sequencing and transfection of HEK293 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.

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 -20ºC if the culture is not purified the same day. Do not store culture at 4ºC over night.
Low Yield	Low copy-number plasmid.	Increase culture volume (up to 10 mL for Minipreps, 100mL for Midipreps, 200 mL for Maxipreps and 3L for Megapreps). Increase the volume of buffer A1, B1, C1 and ethanol proportionally with the ratio of 1:1:1.2:1.2.
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 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.

Vacuum manifold method

From step 7 on page 5.

1. Pull out the plunger of the DNA column and insert the column to the manifold with the vacuum off. Transfer the lysate/ethanol mix to the column and turn on the vacuum till all sample passes through the column.

2. Add **10 mL DNA Wash Buffer** to the column and allow the liquid to pass through the column. Continue vacuum for 1 min. Turn off the column and pull the column out from the manifold.

3. Use the plastic wrench (Provided) to detach the end component from the midiprep column and insert it into a 1.5 ml eppendorf tube.

4. Spin the column at 13,000- 15,000 rpm (Max speed) for 2 min. Decant the flow through and put the column back to the tube. Spin the column at max speed for 2 min. Transfer the column to a new eppendorf tube.

5. Transfer the column to a new 1.5 mL tube and add **0.2 mL Elution Buffer** to the column for a second elution. The first elution yields 60-70% of the DNA while the second elution yields another 20-30% of the DNA bound to the column.