Contents

Important Notes:

Plasmid Copy Number: 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**	**Copy Numbers**	**Expected Yield (μg /200 mL)**
pSC101	pSC101	5	10-15
pACYC	P15A	10-12	20-25
pSuperCos	pMB1	10-20	20-40
pBR322	pMB1	15-20	30-40
pGEM®	Muted pMB1	300-400	400-500
pBluescript®	ColE1	300-500	400-600
pUC	Muted pMB1	500-700	600-1200

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 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 use product number PD1711.

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 buffers result 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 C1.

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.

Kit Contents

Catalog Number	PD1612-01
Buffer A1	180 mL
Buffer B1	180 mL
Buffer C1	220 mL
EndoClean Buffer	10 mL
Elution Buffer	30 mL
DNA Wash Buffer	2x50 mL
DNA Unit	2
Filter Unit	2
Filter Unit Replacement Cup	2
RNase A	650 μL

*Buffer C1 contains acetic acid, wear gloves and protective eyewear when handling.

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.
- DNA Wash Buffer: Add 200 mL 100% ethanol to each bottle before use.
- Keep the cap tightly closed for buffer B1 after use.
- Ensure the availability of centrifuge capable of 13,000 rpm.

Materials supplied by users:

- 100% ethanol.
- Vacuum system.
- 250 mL or 500 mL bottle (Corning# 430282) or 1,000 mL bottle (#430518) or equivalent.
- 50 mL conical tubes.

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.

EZgene ™ Plasmid Megaprep

1. **Inoculate 500 - 800 mL LB containing appropriate antibiotic with 500 µl fresh starter culture. Grow at 37°C for 14-16 h with vigorous shaking.** Harvest 500 mL overnight bacterial cells by centrifugation at 5,000 x g for 10 minutes at room temperature. Decant or aspirate medium and discard.
   Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 2 ml LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 h with vigorous shaking (~250 rpm).

2. Resuspend the bacterial pellet in ***60 mL Buffer A1*** (Add RNase A into ***Buffer A1*** before use). Pipet or vortex till the bacterial pellet dispersed thoroughly (Complete resuspension is critical for optimal yields).

3. **Add *60 mL Buffer B1*, mix thoroughly by inverting 10 times with mild 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 37°C to dissolve before use.

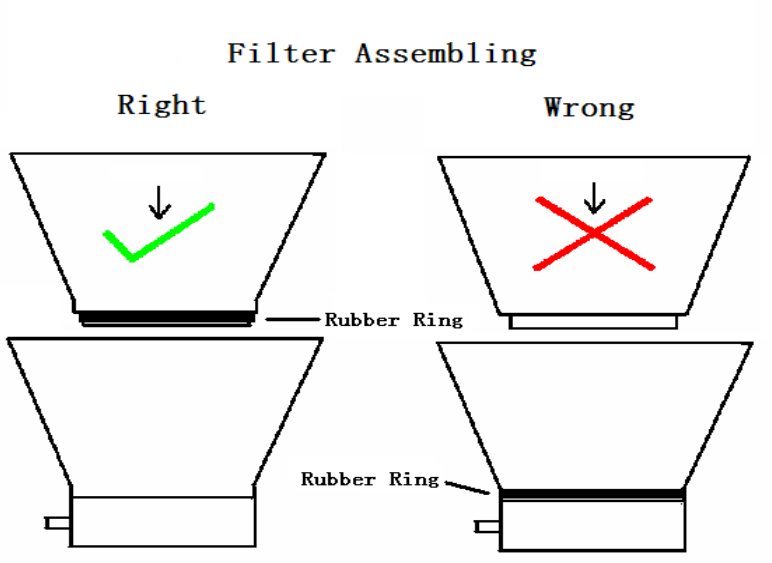
4. Add ***12 mL Buffer C1*** and mix immediately by inverting 5 times till a flocculent white precipitate forms. Mix the lysate well by sharp shaking for 5 times.
   Note: It is critical to mix the lysate well, if the mixture still appears conglobated, brownish or viscous, more mix is required to completely neutralize the solution.

5. Incubate the mixture at room temperature for 10 minutes. Add ***60 mL Buffer C1***, mix by inverting 5 times and set for 2 minutes.

6. Attach the 2-layer filter unit to a **sterile** 500 mL or 1000 mL standard bottle (Corning# 430518 or 430282 or equivalent) and screw tight. Connect the unit to a pump-driven vacuum system.

7. Transfer the clear lysate from the bottom of the mixture (use a 50 mL serological pipet) to the filter unit. Stand by for 2 minute and turn on the vacuum.

   <u>Note 1</u>: Use a 50 mL serological pipet to transfer the relatively clear lysate from the bottom of the lysate bottle to the filter unit. This will speed up the flow rate of the filter unit. Pour the remaining white precipitates to the filter unit when most of the lysate has been filtered through. Normally around 180 mL lysate can be filtered through the filter unit within 10 minutes.
   <u>Note 2</u>: If the flow through gets too slow, turn off the vacuum and wait for 1 minute. Carefully detach the upper filter cup and replace it with the replacement cup. Assemble the unit as instructed in page 1. Pour the lysate from the original cup to the replacement cup. Turn on the vacuum and filter the rest of the lysate.



8. When most of the lysate has been filtered through the unit, turn off the vacuum, wait for 1 minute, detach the unit and discard the upper filter cup including the rubber rings. **Note: The DNA is in the solution in the collecting bottle.**

9. Connect the DNA unit to a clean 500 mL bottle and screw tight. Connect the DNA binding unit to the vacuum with the vacuum off. Add ***60 mL 100% ethanol*** to the lysate bottle. Mix well by vortexing for 5 seconds and pour the lysate/ethanol mixture to the DNA binding unit and turn on the vacuum.

10. Pour the rest of the lysate/ethanol mixture into the DNA binding unit. When all the lysate pass through the DNA binding unit, vacuum for another 2 minutes.

11. Add ***80 mL DNA Wash Buffer*** evenly to the DNA membrane and vacuum for 1 minute. Turn off the vacuum, wait for 1 minute, and discard the liquid waste in the bottle. Reconnect the bottle to DNA binding unit.

12. Add ***40 mL 100% ethanol*** to the DNA membrane and vacuum for 1 minute. Turn off the vacuum, wait for 1 minute, and discard the liquid waste in the bottle. Reconnect the bottle to DNA binding unit.

13. Turn on the vacuum for 20 minutes at maximum force (It is critical to dry the residual ethanol for optimal yield).

14. Turn off the vacuum, wait for 1 minute, and replace the 500 mL or 1,000 mL bottle with a sterile 50 mL conical tube, screw tight.

15. Add ***10 mL Elution Buffer*** evenly to the membrane and incubate for 2 minutes. Turn on vacuum to elute DNA. Typically 5-6 mL of DNA containing solution can be collected. Turn off the vacuum. Optional: Add the eluted DNA back to the mega column for a second elution.
    Note: The first elution normally yields 60-70% of the DNA while a repeated elution yields another 20% of the DNA.

Note 1: The DNA is ready for down stream applications such as cloning or transfection of HEK293 cells.

Note 2: It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Endotoxin Removal Procedure

This protocol is designed to remove endotoxin after the plasmid DNA is purified.

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..

6. Carefully transfer the upper clear layer solution to a 50 mL tube.

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

8. Follow standard DNA precipitation protocol and resuspend DNA in with Elution Buffer.

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

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 Buffer 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.