Plasmid Maxiprep Kit

Contents

Important Notes:

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 3 to 5 times. 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	

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 PD1713.

Optimal cell mass (OD_{600} x mL of Culture): This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) for 12 -16 hours to a 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 biomass over lysis buffer results in low DNA yield and purity. The maxi column has an optimal biomass of 400-500. For example, if the OD_{600} is 2.5, the optimal culture volume should be 150 to 200 mL.

Introduction

Key to the kit is our proprietary DNA binding system that allows the highly 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 12 months from the date of purchase.

Kit Contents

Catalog Number	D1511-01	D1511-02
Preps	10	25
Maxi Column	10	25
Buffer A1	120 mL	260 mL
Buffer B1	120 mL	260 mL
Buffer C1	140 mL	320 mL
DNA Wash Buffer	50 mL	2 x 50 mL
EndoClean Buffer	10 mL	25 mL
Elution Buffer	25 mL	60 mL
RNase A	420 µL	900 µL

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 200 mL 100% ethanol to each DNA Wash Buffer.
- 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, and or vacuum manifold.
- Megaprep: Vaccum system, 500 mL bottle (Corning# 430282) or 1,000 mL bottle (#430518) or equivalent.

EZgene ™ Plasmid Maxiprep Protocol

1. **Inoculate 150 -200 mL LB containing appropriate antibiotic with 100 µL fresh starter culture. Grow at 37°C 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 200 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 10 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 10 mL Buffer B1, mix thoroughly by inverting 10 times with slightly shaking. Incubate for 5-10 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.

5. **Add 4 mL Buffer C1, mix completely by inverting vigorously 10 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 10-15 min at **room temperature**! Transfer the cleared lysate to a 50 ml conical tube. Add **8 mL Buffer C1** and mix well. Proceed to step 7.

ezFilter syringe: Incubate the lysate at room temperature for 5 min. Tape the barrier of the syringe to a 50 mL conical tube. Add **8 mL Buffer C1** to the lysate and pour the lysate into the barrel of the filter syringe. Incubate at room temperature for 5 min. 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 10 mL absolute ethanol (96-100%) to the cleared lysate. Mix well by inverting vigorously 5-10 times. .

8. Transfer 20 mL of the lysate/ethanol mixture into a DNA column with the collection tube. Centrifuge at 5,000 g for 2 min. Discard the flow-through. Put the column back to the collection tube. Load the remaining mixture to the column and centrifuge for 2 min. Discard the flow-through. Put the column back to the collection tube.

9. Add 18 mL DNA Wash Buffer into the column, centrifuge at 5,000 g for 2 min. Discard the flow-through. Put the column back to the collection tube.

10**. Centrifuge the column at 5,000 g for 20 min. Transfer the column into a clean 50 ml tube.** ***Important:*** Removal of ethanol is critical for DNA elution. If swing bucket centrifuge can not reach the speed, use the fixed-angle high speed centrifuge.

11**. Add 1-2 mL Elution Buffer to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 5,000 g for 5 min.** If higher DNA concentration is needed, use less elution buffer.

12. **Optional:** Add the eluted DNA back to the column and spin at 5,000 x g for 5 min. The first elution normally yields about 60-70% of the DNA and the second elution yields another 20-30% of the DNA.

Note: The DNA is ready for down stream applications such as cloning or 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.

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

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 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.

Endotoxin 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 µL 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.