EZgene ™ Plasmid Maxiprep Vacuum Manifold

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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	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^R	Muted pMB1	300-400	400-500
pBluescript^R	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, 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 systems that allow 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 4ºC 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	D1512-01	D1512-02
Maxi Columns	10	25
Syringe Filters	10	25
Buffer A1	120 mL	260 mL
Buffer B1	120 mL	260 mL
Buffer C1	140 mL	320 mL
RNase A	12 mg	25 mg
Elution Buffer	15 mL	50 mL
EndoClean Buffer	N/A	N/A

Caution: 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 50ºC 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:

- 70% ethanol and 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 capable of generating 200-300 mbar.
- Megaprep: Vaccum system, 500 mL bottle (Corning# 430282) or 1,000 mL bottle (#430518) or equivalent.

EZgene ™ Plasmid Maxiprep Vacuum Protocol

1. Inoculate 150 -200 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. Grow at 37°C for 8 h with vigorous shaking (~250 rpm) and use the culture as starter culture. *Warning:* Do not use more than 250 ml culture. Need to scale up buffers if processing more than 250 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 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 50ºC to dissolve before use.

5. Add 4 mL Buffer C1, mix completely by inverting 10 times and sharp shaking for 3 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 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube. Add 8 mL Buffer C1 and mix well. Go to step 8.

ezFilter syringe: Allow the cell lysate to sit for 8 minutes. Add 8 mL Buffer C1 and mix well by inverting 2 times. Set for 1 min. The white precipitates should float to the top. Set the filter syringe barrel at a column holder or tape the syringe barrel to a clean 50 mL tube. Transfer the relatively clear lysate from the bottom of the tube to the barrel of the syringe, avoid the major precipitations. Gently insert the plunger to expel the cleared lysate to the clean 50 mL tube.

7. Attach the maxi column to a vacuum manifold according to the manufacturer's instruction.

8. Add 10 mL absolute ethanol (96-100%) to the clear lysate. Mix well by sharp shaking for 2 times.

9. Immediately transfer 20 mL lysate/ethanol mixture to the column, apply vacuum to allow sample to pass through the column. Transfer the remaining lysate/ethanol mixture to the column till all the sample has been passed through the column.

10. Add 10 mL 70% ethanol into the column and allow the liquid to pass through the column by vacuum. Repeat once.

Turn off the vacuum and decant the flow-through liquid inside the manifold. Connect the manifold back to the vacuum system.

11**.** Turn on the vacuum to dry the column for 15-20 minutes. *This step removes ethanol residues.*
Note*: Increase the time to 25 minutes if over 10 samples are being processed.*
Note: Alternatively, the column can be dried by centrifugation in a swing-bucket rotor for 10 minutes.

12**.** Turn off the vacuum and detach the column from the manifold, tap the column tip on layers of clean paper towel. Wipe off any trace ethanol inside the column. Transfer the column to a clean 50 mL conical tube.

13. Add *1.0-2.0 mL Elution Buffer* to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 5,000 x g for 1 min. Add the eluted DNA back to the column, incubate for 1 min, and then elute the DNA by centrifugation at 5,000 x g for 3 min. The first elution normally yields 60-70% of the DNA bound. Add the eluted DNA back to the column and centrifuge at 5,000 x g for 3 min. The second elution yields another 20% of the DNA bound.

14. Two elution give rise to maximum DNA yield. Use less elution buffer if higher DNA concentration is desired. For maximum DNA yield, elute twice with 2 mL of Elution Buffer, precipitate the DNA, and resuspend DNA in Elution Buffer.

Note: The DNA is ready for downstream 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.

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 overnight.
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 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 eluting 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 **0.1 volume** of **EndoClean buffer** to the plasmid sample in a 1.5 mL sterile tube. (For example, add 0.1 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 37-55 °C water baths for about 2 minutes and the solution shall be turbid.

5. Centrifuge at top speed at **room temperature** for 3 minutes.

6. Carefully transfer the upper clear layer solution to 2.0 mL tube.

7. Precipitate plasmid DNA with 0.1 volume of 3 M KAc (pH 5.2) and 0.7 volume of 100% isopropanol. Mix well.

8. Spin at 13,000 rpm for 10 min. Decant and add 500 µL 70% ethanol. Centrifuge at 13,000 rpm for 5 min. Decant.

9. Dry the DNA in a speedvac for 5-10 min or airdry the sample at a TC hood till DNA is completely dry. Resuspend the DNA in Endofree water