Endofree Palsmid Midiprep Kit

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	3-5
pACYC	P15A	10-12	10-15
pSuperCos	pMB1	10-20	10-30
pBR322	pMB1	15-20	15-30
pGEM^{R}	Muted pMB1	300-400	100-120
pBluescript^{R}	ColE1	300-500	100-150
pUC	Muted pMB1	500-700	100-250

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 buffers result 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 high efficient binding of DNA to our ezBind^{TM} matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily eluted with Elution Buffer. Our proprietary endotoxin removal buffer, Buffer RET, is designed to remove endotoxin by a single washing step without tedious phase partitioning steps. The purified plasmid DNA, with endotoxin level less than 0.1 EU per µg of DNA, is ready for transfetion of endotoxin sensitive cell lines and microinjections.

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	D1420-01	D1420-01	PD1420-02
Maxi Columns	2	10	25
Syringe filters	2	10	25
Buffer A1	10 mL	30 mL	70 mL
Buffer B1	10 mL	30 mL	70 mL
Buffer N3	5 mL	20 mL	40 mL
Buffer RET	10 mL	30 mL	70 mL
DNA Wash Buffer*	12 mL	12 mL	50 mL
RNase A	50 µL	100 µL	250 µL
Endofree Elution Buffer	1.5 mL	15 mL	30 mL

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.
- DNA Wash Buffer: Add 48 mL (PD1420-00 and PD1420-01) and 200 mL (PD1420-02) 96%-100% ethanol to each bottle before use.
- Ensure the availability of centrifuge capable of 13,000 rpm or use the syringe filters.
- **Carry out all centrifugations at room temperature.**

Materials supplied by users:

- 100% ethanol.
- Midiprep: 50 mL centrifuge tubes and high speed 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.

ezFlow Endofree Plasmid Midiprep II Protocol

1. Inoculate 50 mL LB containing appropriate antibiotic with 50 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. Note: The ezFlow flow system yields less plasmid DNA as compared to the regular midiprep due to the loss of DNA during endotoxin removal procedure. To increase the yield, use high end of culture volume. Allow the cell density to get above an OD600 of greater than 2.5.

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

4. Add 2.5 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.

5. Add 1.5 mL Buffer N3, 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. Transfer the lysate to the barrier of a syringe filter and put the syringe inside a 50 mL conical tube for 10 min at room temperature. Gently insert the plunger to the barrier to expel the clear lysate into the tube. Connect the midi column to a manifold according to the manufacturer's instruction.
Note: Lysate also can be cleared by centrifugation: transfer the lysate from step 5 into a high speed centrifuge tube and centrifuge at 13000 rpm for 15 minutes. Transfer the clear lysate into a clean 50 mL tube. Proceed to next step.

7. Add 2.5 mL Buffer RET and 2.5 mL ethanol to the sample, mix well and transfer 20 mL of the sample to a DNA column with a collection tube. Centrifuge at 5,000 g for 5 minutes at room temperature. Discard the flow through liquid and put the column back to the column. Process the remaining lysate as described if any.

8. Add 5 mL DNA Wash Buffer to the column and Centrifuge at 5,000 g for 5 minutes at room temperature. Discard the flow through liquid and put the column back to the column.

9. Centrifuge the column for 15 minutes at maximum speed.
Note: This step removes residual ethanol for optimized elution in the next step.

10. Transfer the column to a clean 50 mL conical tube and add 0.5 mL Endofree Elution Buffer to the center of the column. Incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 5,000 x g for 5 min.

11. Optional: Add the eluted DNA back to the column and spin at 5,000 x g for 3 min. Two elutions give rise to maximum DNA yield.

Note: The DNA is ready for down stream applications such as transfection of endotoxin sensitive cell lines and microinjections.

Note: The first elution normally yields 60-70% of DNA and the second elution yields another 20-30%.

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 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.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

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For technical support or learn more product information, please contact us at (858) 603-3219 or visit our website at www.biomiga.com