Endofree Plasmid Maxiprep Kit

Important Notes...1
Introduction...2
Storage, Stability and Kit Contents 2
Before Starting...3
Endofree Plasmid Maxiprep Protocol 4
Trouble Shooting Guide...7
Limited Use and Warranty...8

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.

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™ 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 4ºC once RNase A is added. Buffer ERT should be stored at -20ºC. All other materials can be stored at room temperature. The Guaranteed shelf life is 12 months from the date of purchase.

Kit Contents

Size	PD1520-00	PD1520-01	PD1520-02
Maxi Columns	2	10	25
Buffer A1	30 mL	120 mL	260 mL
Buffer B1	30 mL	120 mL	260 mL
Buffer N3	10 mL	40 mL	80 mL
Buffer RET	30 mL	120 mL	260 mL
DNA Wash Buffer	12 mL	50 mL	2x50 mL
RNase A	110 µL	400 µL	1000 µL
Endofree Elution Buffer	3mL	15 mL	50 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 RNase A to Buffer A1. Mix well before use. Store at 4°C.
- Buffer B1 precipitates below room temperature, it is critical to warm up the buffer at 37ºC to dissolve the precipitates before use.
- Keep the cap tightly closed for buffer B1 after use.
- Ensure the availability of centrifuge capable of 5,000 x g.
- Carry out all centrifugations at room temperature.
- Add 48 mL (PD1520-00) or 50 mL (PD1520-01 and PD1520-02) to each DNA Wash Buffer before use.

Materials supplied by users:

- 100% ethanol
- 50 mL conical tubes.
- Centrifuge with swing bucket rotor and centrifuge tubes.

Endofree Plasmid Maxiprep 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.

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 completely.

3. Add 10 mL Buffer A1 to bacterial pellet and completely resuspend by vortexing or pipetting. Transfer the lysate to a 50 mL conical tube. 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.

5. Add 3 mL Buffer N3, mix completely by inverting 10 times and sharp shaking for 3 times. It is critical to mix the solution well.

Note: if the mixture still appears conglobated, brownish or viscous, more mixing is required to completely neutralize the solution.

6. Centrifuge the sample at 5,000 g for 10 min at room temperature. Transfer the clear lysate to a new 50 mL conical tube.

7. Add 10 mL Buffer RET and 10 mL ethanol and mix well by vortexing for 5 seconds.

8. Transfer 20 mL of sample to a DNA maxi column, and centrifuge at 5,000 rpm for 5 min. Discard the flow through and put the column back to the collection tube. Process the remaining lysate as described. Discard the flow through and put the column back to the collection tube.

9. Add 10 mL DNA Wash Buffer to the column and centrifuge at 5,000 rpm for 5 min. Discard the flow through and put the column back to the collection tube.

10. Centrifuge the column for 15 min at 5,000 rpm. Carefully transfer the column to a clean 50 mL conical tube.

11. Add 1.5-2 mL endofree 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 5 min.

12. Add the eluted DNA back to the column and centrifuge at 5,000 x g for 5 min for a second elution.

Note: The first elution yields approximately 70% of the DNA. The second elution yields another 20% of the DNA. The maxiprep column has high DNA binding capacity (~ 2.5 mg).

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

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.

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