Endofree Plasmid Miniprep Kit

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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 / 1 mL)
pSC101	pSC101	5	0.1-0.2
pACYC	P15A	10-12	0.4-0.6
pSuperCos	pMB1	10-20	0.4-1
pBR322	pMB1	15-20	0.6-1
pGEM^{R}	Muted pMB1	300-400	6-7
pBluescript^{R}	ColE1	300-500	6-8
pUC	Muted pMB1	500-700	8-12

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^{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	PD1222-00	D1220-01	D1220-02
Mini Columns	4	50	250
Buffer A1	2 mL	25 mL	125 mL
Buffer B1	2 mL	25 mL	125 mL
Buffer N3	400 µL	10 mL	50 mL
Buffer RET	2 mL	25 mL	125 mL
Buffer KB	3 mL	25 mL	125 mL
DNA Wash Buffer	1 mL	12 mL	50 mL
RNase A	10 µL	90 µL	420 µL
Endofree Elution Buffer	500 µL	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 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 50°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- Add 48 mL (1222-01) or 200 mL (1222-02) ethanol to DNA Wash Buffer before 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 microtubes.
- 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 Miniprep Protocol

1. Inoculate 5-12 mL LB containing appropriate antibiotic with a freshly streaked colony. Grow at 37°C for 14-16 h with vigorous shaking.
   Note: Always pick up single colonies that are freshly streaked from glycerol stock or freshly transformed.
   Note: Using colonies that have been stored at 4°C may result in low plasmid yield.

2. Harvest the bacterial by centrifugation at 12,000 rpm for 1 min at room temperature. Pour off the supernatant.

   Note: The culture can be centrifuged at 6,000 rpm in a 15 mL conical tube for 5 minutes if high speed centrifuge tubes are not available. Alternatively, the cultures can also be spin down in multiple 2.0 mL tubes.

   Note: Complete removal of residue medium is critical for bacteria lysis in the next step.

3. Add 450 µL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting.
   Note: Complete resuspension is critical for optimal yield.

4. Add 450 µL 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.

5. Add 100 µL 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. Centrifuge the sample at 13,000 rpm for 10 min at room temperature. Transfer the cleared lysate to a 1.5 ml microtube. Add 450 µL Buffer RET, mix well by vortexing for 5 seconds.

7. Transfer 700 µL of sample to a DNA column with a collection tube and centrifuge at 12,000 rpm for 20 seconds. Discard the flow-through and put the column back to the collection tube. Process the remaining sample as described.

8. Add 400 µL Buffer KB to the column and centrifuge at 12,000 x g for 30 seconds. Discard the flow-through and put the column back to the collection tube.

9. Add 500 µL DNA Wash Buffer to the column and centrifuge at 12,000 x g for seconds. Discard the flow-through and put the column back to the collection tube.

10. Centrifuge the empty column at maximum speed for 1 minute. Note: This step removes residual ethanol for optimized elution in the next step.

11. Transfer the column to a clean 1.5 mL tube and add 50-100 µL Endofree Elution Buffer to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 12,000 rpm for 1 min.

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

12. Optional: Add the eluted DNA back to the column and centrifuge at 12,000 rpm for 1 min.

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, N3, RET 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.

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) 597-0602 or visit our website at www.biomiga.com