Endofree ezFilter Plasmid Maxiprep 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	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 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	**PD1522-01**	**PD1522-02**
Maxi Columns	10	25
Syringe Filters	10	25
Buffer A1	120 mL	260 mL
Buffer B1	120 mL	260 mL
Buffer N3	70 mL	200 mL
RET	120 mL	260 mL
DNA Wash Buffer	50 mL	2x50 mL
RNase A	400 µL	900 µL
Endofree Elution Buffer	30 mL	60 mL

*Buffer RET contains chaotropic salt, Buffer N3 contains acetic acid, wear gloves and protection eye wear when handling these buffers.

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 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 10,000 rpm if perform centrifugation protocol.
- Ensure the availability of centrifuge capable of 5,000 x g if perform vacuum manifold protocol.
- Carry out all centrifugations at room temperature.

Materials supplied by users:

- 100% ethanol
- 50 mL conical tubes.
- Centrifuge with swing bucket rotor and centrifuge tubes.
- Vacuum manifold.
- Pump driven vacuum system capable if generating 200-600 mBar.

Vacuum manifold Protocol

1. Inoculate 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. 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.

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 4 mL Buffer N3, mix completely by inverting 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. Spin the sample at 5,000 g for 2 min at room temperature. Tape the barrier of a syringe to a 50 mL conical tube set in a rack. Using a serological pipet to transfer most of the lysate, avoid only a small portion of the major precipitates, to the barrier of the syringe. Let it set for 5 min. Gently insert the plunger to the barrier to expel the clear lysate into the tube.

Note: The cleared lysate should be clear and free of cloudy

precipitates. If it looks turbid or cloudy, add 2 mL of Buffer N3, mix well and filter though another syringe filter. Alternatively, the sample can be centrifuged at 5,000 rpm for 5 minutes, transfer the clear supernatant to a clean 50 mL conical tube.

7. Add 10 mL Buffer RET and 10 mL ethanol and mix well by vortexing for 2 seconds. Insert a maxi column to a manifold according to manufacturer's instruction.

8. Transfer 20 mL of sample to a DNA maxi column, apply vacuum to allow the lysate pass through the column. Process the remaining lysate till all lysate pass through the column. Note: If the flow through gets too slow, spin the column at 5,000 x g in a 50 mL conical tube for 5 minutes.

9. Add 10 mL DNA Wash Buffer to the column and allow the liquid pass through the column.

10. Optional: Add 10 mL DNA Wash Buffer to the column and allow the liquid pass through the column.

11. Turn off the vacuum and decant the flow-through liquid inside the manifold. Connect the manifold back to the vacuum. Turn on the vacuum to dry the column for 15 min.

12. Optional: Turn off the vacuum, transfer the column to a 50 mL conical tube and centrifuge at 5,000 g for 5 minutes.

Note: Wipe off any ethanol residual inside the column with kimwipe if any. Step 11 and 12 remove residual ethanol for optimized elution in the next step. Swing-bucket type rotor is preferred for centrifugation.

13. Transfer the column to a clean 50 mL conical tube and 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.

14. Add the eluted DNA back to the column and centrifuge at 5,000 x g for 5 min.

Centrifuge protocol:

1. From step 5 on Page 4, spin the lysate at 10,000 rpm for 20 min. Transfer the clear lysate to clean 50 mL tube.
2. Add 10 mL Buffer RET, mix well by vortexing for 5 seconds.
3. Apply 20 mL of sample to a DNA maxi column with the collection tube, spin at 5,000 rpm for 5 min. Discard the flow through and put the column back to the collection tube. Repeat loading the remaining sample.
4. Add 10 mL of DNA Wash Buffer and spin at 5,000 rpm for 5 min. Discard the flow through and put the column back to the collection tube. Repeat once.
5. Spin the maxi column at 5,000 rpm for 10 min to dry the column.
6. Transfer the column to a clean 50 mL tube and add 1.5 – 2 mL of Elution Buffer. Spin at 5,000 rpm for 5 min to elute the DNA.
7. Optional: Add the eluted DNA back to the column for a 2nd elution.

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