EasyPrep™ EndoFree Plasmid Midiprep Purification Protocol

PD03-21, PD03-22
PD03-31, PD03-32

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Trouble shooting guide

Low Yield	Poor Cell lysis	Resuspend the pellet well prior adding buffer B1. Mix the lysate well after adding B1. Make fresh buffer B1 if the cap had not been closed tightly.
	Bacterial culture overgrown or not fresh	Always start a culture from a fresh single colony. Grow bacterial 12-16 hours. If the culture is not used the same day, spin down the culture and store the pellet at –20°C.
	Low-copy-number plasmid	If possible, change the host bacteria strain to high-copy-number ones. Follow instructions on Page 10.
No DNA	Plasmid lost in Host *E.coli*	Antibiotics lost activity. Prepare fresh agar plates and add antibiotics to medium just before culture. Start from a fresh colony.
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 min after adding Buffer B1.
RNA contamination	RNase A not added to Buffer A1	Add RNase A to Buffer A1:100 µl RNase A (30mg/ml) for 30 ml 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 on the membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.

Table: Other related products

Catalog #	Product Name	Preps
PD01-01	Plasmid miniprep kit	50
PD01-02	Plasmid miniprep kit	250
PD02-01	Plasmid miniprep-II kit	50
PD02-02	Plasmid miniprep-II kit	250
PD02-11	EndoFree Plasmid miniprep-II kit	50
PD02-12	EndoFree Plasmid miniprep-II kit	250
PD03-01	Plasmid midiprep kit	10
PD03-02	Plasmid midiprep kit	25
PD03-11	ezFilter Plasmid midiprep kit	10
PD03-12	ezFilter Plasmid midiprep kit	25
PD04-01	Plasmid midiprep-II kit	10
PD04-02	Plasmid midiprep-II kit	25
PD04-11	ezFilter Plasmid midiprep-II kit	10
PD04-12	ezFilter Plasmid midiprep-II kit	25
PD04-21	EndoFree plasmid midiprep-II kit	10
PD04-22	EndoFree plasmid midiprep-II kit	25
PD04-31	EndoFree ezFilter plasmid midiprep-II kit	10
PD04-32	EndoFree ezFilter plasmid midiprep-II kit	25
PD05-01	Plasmid maxiprep kit	10
PD05-02	Plasmid maxiprep kit	25
PD05-11	ezFilter Plasmid maxiprep kit	10
PD05-12	ezFilter Plasmid maxiprep kit	25
PD05-21	EndoFree plasmid maxiprep kit	10
PD05-22	EndoFree plasmid maxiprep kit	25
PD05-31	EndoFree ezFilter plasmid maxiprep kit	10
PD05-32	EndoFree ezFilter plasmid maxiprep kit	25
PD06-01	ezFilter Plasmid megaprep kit	2
PD06-02	ezFilter Plasmid megaprep kit	10
PD06-11	EndoFree ezFilter Plasmid megaprep kit	2
PD06-12	EndoFree ezFilter Plasmid megaprep kit	10

Introduction

EasyPrep™ EndoFree Plasmid Midiprep Kit provides a simple, fast and cost effective method to purify plasmid DNAs from bacterial culture, when amount of the plasmid DNA needed is about 200 µg. The kit is especially convenient to use when multiple plasmids need to be purified at the same time, as the use of traditional commercial kits is time-consuming.

Key to the kit is our proprietary DNA binding systems that allow the high efficient DNA binding 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 transfection of endotoxin sensitive cell lines and microinjections.

Important Notes

1. **Copy numbers:** The yield of plasmid DNA using the Midiprep kit depends on many factors. The origin of the replication is one of them. Samples of high-copy-number and low-copy-number plasmids are shown in the table. 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 accordingly.

Plasmid	Origin	High copy	Low copy
pACYC	P15A		10-12
pSC101	pSC101		5
pSuperCos	pMB1		10-20
pBR322	pMB1		15-20
pUC	Muted pMB1	500-700	
pGEM®	Muted pMB1	300-400	
pBluescript®	ColE1	300-500	

2. **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.

3. **Bacterial culture**: For maximal yields, the culture should be in late log or early stationary phase of bacteria growth. If the culture overgrows, you will be harvesting more dead bacteria than live cells, which leads to genomic DNA contamination. If the culture is undergrown, then of course, yields are lower than expected. In practice:

a. A day before the bacteria culture, streak a antibiotic containing agar plate with glycerol stock and incubate at 37°C overnight.

b. Pick up a single colony and inoculate into 1-2 ml LB medium containing antibiotics, culture on at 37°C for 6-8 hours with vigorous shaking (300 rpm).

c. Seed 30 µl of the culture to 50-65 ml LB medium containing antibiotics, culture on a shaker at 37°C for 12-16 hours. Use 250 ml flask and shake at 250 rpm speed.

d. OD_{600} of the culture should be 2.5-3.5.

Before Starting

Prepare all components and get all necessary materials ready by examining this manual and become familiar with each step.

- **RNase A**: Spin down RNase A vial briefly. Add the whole tube of RNase A to Buffer A1 and mix well before use. **Store Buffer A1 at 4°C after use**. RNase A final concentration ≥ 100 µg/ml.
- **Buffer B1** could precipitate below room temperature. It is critical to warm up the buffer at 37°C to dissolve the precipitates before use. Keep Buffer B1 bottle cap tightly closed to avoid acidification.
- **Washing Buffer:** Add 100 m (PD03-21,-31) or 200 ml (PD03-22, -32) 96-100% ethanol before use.
- Carry out all centrifugations at **room temperature.**

lysate to incubate for 5 min. Gently insert the plunger to expel the clear lysate.

7. **Add 2.5 mL Buffer RET and mix well by vortexing for 2 seconds.**

8. ***Column preparation:* add 1 ml Column Buffer to the column, incubate for 2-3 min, centrifuge at 3,500 rpm for 3-5 min.** *The column equilibration ensures the column having optimal binding capacity. Especially when the kit is stored over 6 month or the yield declines noticeably*.

9. **Transfer 4.5 ml lysate/ethanol mixture to the column attached to the manifold, apply vacuum to allow sample pass through the column. Transfer the remaining lysate/ethanol mixture to the column till all the sample has been passed through.** Be careful not to overfill the column, the maximum load is 20 ml.

10. **Wash the column with 4 mL DNA Wash Buffer. Discard the flow-through. Repeat once. Leave the vacuum on for 15 min.** It is critical to remove residual ethanol. Increase the time to 20 min if ≥10 samples are processed at same time. The column can also be centrifuged at 3,500 rpm min to remove residual ethanol.

11. **Detach the column from the manifold, wipe off any trace ethanol and put the column to a clean 50 ml conical tube.**

12. **Add 0.2-0.5 ml Elution Buffer to the center of the column. Incubate for 1 min at room temperature. Elute the DNA by centrifugation (swing bucket) at 3,500 rpm for 5 min.** If higher DNA concentration is needed, use less elution buffer.

13. **Add the eluate from last step to the column and elute one more time.** This will increase DNA yield.

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

EndoFree Plasmid Midiprep Protocol

(Vacuum Method)

1. **Inoculate 50-65 ml LB containing appropriate antibiotic with 30 µL fresh starter culture. Grow at 37°C for 14-16 h with vigorous shaking.** Prepare a starter culture by inoculating a single colony from a freshly grown selective plate into 1-2 ml LB medium containing the appropriate antibiotic. Grow at 37°C for 6-8 h with vigorous shaking (~300 rpm).
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 2.5 mL Buffer A1 to bacterial pellet and completely resuspend by vortexing or pipetting. Transfer the lysate to a 50 mL conical tube.** Note: Complete resuspension is critical for optimal yield.
4. **Add 2.5 mL Buffer B1, mix thoroughly by inverting 10 times with slightly shaking. Incubate at room temperature for 5 min to obtain a slightly clear lysate.** Buffer B1 forms precipitation below room temperature. If solution becomes cloudy, warm up at 37-50°C to dissolve before use. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent. Do not incubate longer than 5 min. Over-incubation causes genomic DNA contamination and plasmid damage.
5. **Add 1.25 mL Buffer N3, mix completely by inverting 10. 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. Two options for clearing the lysates:
   a. High speed centrifuge: **Transfer the lysate to a high speed centrifuge tube and centrifuge at 10,000 rpm for 20 min at room temperature! Transfer the cleared lysate to a 15 ml conical tube.**
   b. ezFilter syringe: **Spin the sample at 3,500 rpm for 5 min at room temperature. Place the ezFilter syringe to a centrifuge tube in a tube 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. Allow the lysate**

Kit Contents

Catalog #	PD03-21 PD03-31	PD03-22 PD03-32
Midiprep DNA Columns	10	25
ezFilter	10 (PD03-31)	25 (PD03-32)
Buffer A1	30 ml	75 ml
Buffer B1	30 ml	75 ml
Buffer N3	7.5 ml	15 ml
Buffer RET	30 ml	75 ml
DNA Wash Buffer (5x)*	25 ml	50 ml
Elution Buffer	10 ml	20 ml
Column Buffer	15 ml	30 ml
RNase A	110 µl	250 µl
Manual	1	1

** Add ethanol before use*

Materials supplied by users:

- 96-100% ethanol
- 15 ml conical tubes
- Bench top centrifuge with swing bucket and 5 ml conical tube adapter, speed of the centrifuge can reach ≥ 3,500 rpm.

Limited use and warranty

This product is warranted to perform as described in its labeling and in Bioland'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 Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland 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.

EndoFree Plasmid Midiprep Protocol

(Centrifuge Method)

1. **Inoculate 50-65 ml LB containing appropriate antibiotic with 30 µL fresh starter culture. Grow at 37°C for 14-16 h with vigorous shaking.** Prepare a starter culture by inoculating a single colony from a freshly grown selective plate into 1-2 ml LB medium containing the appropriate antibiotic. Grow at 37°C for 6-8 h with vigorous shaking (~300 rpm).
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 2.5 mL Buffer A1 to bacterial pellet and completely resuspend by vortexing or pipetting. Transfer the lysate to a 50 mL conical tube.** Complete resuspension is critical for optimal yield.
4. **Add 2.5 mL Buffer B1, mix thoroughly by inverting 8-10 times. Incubate at room temperature for 5 min to obtain a slightly clear lysate.** Buffer B1 forms precipitation below room temperature. If solution becomes cloudy, warm up at 37-50°C to dissolve before use. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent. Do not incubate longer than 5 min. Over-incubation causes genomic DNA contamination and plasmid damage.
5. **Add 0.62 mL Buffer N3, mix completely by inverting 8-10 times. It is critical to mix the solution well. I**f the mixture still appears conglobated, brownish or viscous, more mixing is required to completely neutralize the solution.
6. Two options for clearing the lysates:
   a. High speed centrifuge: **Transfer the lysate to a high speed centrifuge tube and centrifuge at 10,000 rpm for 20 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube.**
   b. ezFilter syringe: **Spin the sample at 3,500 rpm for 5 min at room temperature. Place the ezFilter syringe to a centrifuge tube in a tube 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. Allow the lysate to incubate for 5 min. Gently insert the plunger to expel the clear lysate.**
7. **Add 2.5 mL Buffer RET and mix well by inverting 8-10 times.**
8. ***Column preparation:* add 1 ml Column Buffer to the column, incubate for 2-3 min, centrifuge at 3,500 rpm for 3-5 min.** *The column equilibration ensures the column having optimal binding capacity. Especially when the kit is stored over 6 month or the yield declines noticeably*.
9. **Transfer 4.5 mL of sample to a DNA midiprep column with a 50 mL conical tube and centrifuge at 3,500 rpm for 3-5 min. Discard the flow-through and process the remaining sample by repeating.**
10. **Add 4 mL DNA Wash Buffer to the column and centrifuge at 3,500 rpm for 3-5 min. Discard the flow-through. Repeat once.**
11. **Centrifuge the column at 3,500 rpm for 10 min to remove residual ethanol.** ***Important:*** Removal of residual ethanol is critical for DNA elution. If swing bucket centrifuge can't reach the speed, use the fixed-angle high speed centrifuge.
12. **Transfer the column to a clean 50 mL conical tube and add 0.2-0.5 mL Elution Buffer to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 3,500 rpm for 5 min.** Use less elution buffer if higher DNA concentration is desired.
13. **Add the eluted DNA back to the column and centrifuge at 3,500 rpm for 5 min.** The first elution normally yields approximately 70% of the DNA. The second elution yields another 20% of the DNA bound to the column.

The DNA is ready for downstream applications such as transfection of endotoxin sensitive cell lines, primary cell lines and microinjections.