EasyPrep™ Plasmid Midiprep Manual

Catalog#: PD03-01, PD03-02, PD03-03
PD03-11, PD03-12, PD03-13

Bioland

For research use only

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Table of Contents

Introduction

EasyPrep™ Plasmid Midiprep Kit provides a simple, fast and cost effective method to purify plasmid DNAs from bacterial culture. The kit applies DNA-binding-specific matrix, which allows the high efficient binding of DNA, while proteins and other cellular components are easily removed during washing. Instead of traditional resin columns, the kit uses spin columns, which dramatically reduce the processing time from a couple of hours to within one hour. Specifically, sample loading and washing step takes only 5 minutes, respectively. This is especially beneficial when multiple plasmids need to be purified at the same time, all could be done in the same spin.

The yield of the high-copy plasmid from this kit is about 200 µg. Plasmid DNAs purified from this kit can be used directly for downstream applications such as transfection, cloning, sequencing, as well as *in vivo* applications.

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
pSA301	pSA301		5
pSuperCos	pMA2		10-20
pBR322	pMA2		15-20
pUC	Muted pMA2	500-700	
pGEM®	Muted pMA2	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, HA201, 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, more dead bacteria will be harvested 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 25 µl of the culture to 50 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 A2** could precipitate below room temperature. It is critical to warm up the buffer at 37-50°C to dissolve the precipitates.
- Keep Buffer A2 bottle cap tightly closed to avoid acidification.
- Prepare 70% ethanol
- Carry out all centrifugations at **room temperature.**

Kit Contents

Catalog #	PD03-01 PD03-11	PD03-02 PD03-12	PD03-03 PD03-13
Midiprep Columns	10	25	50
EasyFilter	10 (PD03-11)	25 (PD03-12)	50 (PD03-13)
Buffer A1	30 ml	80 ml	160 ml
Buffer A2	30 ml	80 ml	160 ml
Buffer A3	45 ml	100 ml	250 ml
Elution Buffer*	10 ml	20 ml	30 ml
Column Buffer	15 ml	30 ml	60 ml
RNase A	110 µl	275 µl	540 µl
15ml conical tubes	10	25	50
Manual	1	1	1

*Elution Buffer: 10mM Tris.HCl, 1mM EDTA, pH8.5 (TE buffer, pH8.5).

Materials supplied by users

- 96-100% ethanol
- 15ml conical tubes
- Bench top centrifuge with swing bucket and 50 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.

EasyPrep™ Plasmid Midiprep Protocol

(Centrifuge Method)

1. **Harvest 50-60 ml bacterial by centrifugation at 3,500rpm for 10 min at room temperature. Pour off the supernatant and inverte tube on paper towels to remove residual medium.**
2. **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.*
3. **Add 2.5 ml Buffer A2, mix gently but thoroughly by inverting 5-6 times with slightly shaking. Incubate for 3-4 min to obtain a slightly clear lysate.** *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.*
4. **Add 1 ml Buffer A3, mix immediately but thoroughly by inverting 5-6 times. Don't vortex.** *It is important to mix the solution well, if the mixture still appears conglobated, more mix is required.*
5. Two options for clearing the lysates:
   a. High speed centrifuge: **Transfer the lysate to a high speed centrifuge tube and centrifuge at 13,000 rpm (14,000-18,000 g) for 15 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube.**
   b. EasyFilter syringe: **place the EasyFilter syringe to a clean 50 ml conical tube with the plunger removed. Pour the lysate into the filter syringe barrel. Allow the lysate to sit for 5 min. The white precipitates should float to the top. Hold the filter syringe barrel over the 15 ml tube and gently insert the plunger to expel the cleared lysate to the tube. Stop when feel resistance. some of the lysate may remain in the flocculent precipitate.**
6. **Add 2 ml Buffer A3 and 2.5 ml Ethanol (96-100%). Mix well by inverting 5-6 times.**

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

8. **Transfer up to 4.5 ml lysate/ethanol mixture into a DNA column with the collection tube. Centrifuge at 3,500 rpm for 3-5 min. Discard the flow-through.**

9. **Add 4.5 ml 70% ethanol into the column, centrifuge at 3,500 rpm for 3-5 min. Discard the flow-through.**

10. **Repeat Step 9.**

11. **Centrifuge at 3,500 rpm for 5 min.** *This step removes residual ethanol which is critical for DNA elution. Residual ethanol may also interfere subsequent applications.*

12. **Place the column in a sterile 50 ml tube. Add 0.25-0.5 ml Elution Buffer (TE buffer, pH8.5) to the center of the column. Incubate for 1 min. Elute the DNA by centrifugation at 3,500 rpm for 2-5 min.** *If higher DNA concentration is needed, use less elution buffer (i.e. 0.25 ml).*

13. **Add the eluate from last step back to the column, elute one more time.** *This will increase the yield. Alternatively, elute with another 0.25-0.5 ml Elution Buffer into a new 15 ml tube. Two elutions give rise to maximum DNA yield. Store DNA at –20°C.*

The DNA is ready for down stream application such as cloning or transfection. If higher DNA quality and concentration is desired, such as for primary cultured cell or some cell line transfection, proceed to the next step. It's also highly recommended to remove the endotoxin if the DNA is used for primary cultured cell transfection or microinjection.

EasyPrep™ Plasmid Midiprep Protocol

(Vacuum Method)

1. **Harvest 50-60 ml 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.**

2. **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.*

3. **Add 2.5 ml Buffer A2, mix gently but thoroughly by inverting 5-6 times with slightly shaking. Incubate for 3-4 min to obtain a slightly clear lysate.** *Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent. Do not incubate longer than 5 min. Over-incubating causes genomic DNA contamination and plasmid damage.*

4. **Add 1 ml Buffer A3, mix immediately but thoroughly by inverting 5-6 times.** *It is important to mix the solution well, if the mixture still appears conglobated, more mix is required.*

5. Two options for clearing the lysates:

   a. High speed centrifuge: **Transfer the lysate to a high speed centrifuge tube and centrifuge at 13,000 rpm (14,000-18,000 g) for 15 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube.**

   b. EasyFilter syringe: **place the EasyFilter syringe to a clean 50 ml conical tube with the plunger removed. Pour the lysate into the filter syringe barrel. Allow the lysate to sit for 5-10 min. The white precipitates should float to the top. Hold the filter syringe barrel over the 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube. Stop when feel resistance. some of the lysate may remain in the flocculent precipitate.**

6. **Add 2 ml Buffer A3 and 2.5 ml Ethanol (96-100%). Mix well by inverting 5-6 times.**

7. **Column preparation: add 1 ml Column Buffer to the column attached to the vacuum manifold incubate for 2-5 min, switch on vacuum source to draw the solution through the columns completely.** *This step ensures the column having optimal binding capacity. Especially when the kit is stored over 6 month or the yield declines noticeably*.

8. **Transfer up to 4.5 ml lysate/ethanol mixture to the column, switch on vacuum to draw the solution through the columns completely, and then switch off. Apply the remaining lysate/ ethanol mixture.**

9. **Add 4.5 ml 70% ethanol into the column. Switch on vacuum source to draw the solution through the columns completely, and then switch off vacuum source.**

10. **Repeat Step 9, leave the vacuum on for 2 min and then switch off.** *Removal of residual ethanol is critical for DNA elution. Residual ethanol may interfere subsequent applications. Increase the time 5-10 min if ≥10 samples are processed at same time*.

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 0.25-0.5 ml Elution Buffer (TE buffer, pH8.5) to the center of the column, incubate for 1 min, elute the DNA by centrifugation at 3,500 rpm for 2-5 min.** *If higher DNA concentration is needed, use less elution buffer (i.e. 0.25 ml)*.

13. **Add the eluate from last step back to the column, elute one more time.** *This will increase the yield. Alternatively, elute with another 0.25-0.5 ml Elution Buffer into a new 15 ml tube. Two elutions give rise to maximum DNA yield. Store DNA at –20°C.*

The DNA is ready for down stream application such as cloning or transfection. If higher DNA quality and concentration is desired, such as for primary cultured cell or some cell line transfection, proceed to the next step. It's also highly recommended to remove the endotoxin if the DNA is used for primary cultured cell transfection or microinjection.

Low-copy-number Plasmid Midiprep Protocol

1. **Harvest 100-120 ml 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.**

2. **Add 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.*

3. **Add 5 ml Buffer A2, mix gently but thoroughly by inverting 5-6 times with slightly shaking. Incubate for 3-4 min to obtain a slightly clear lysate.** *Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent. Do not incubate longer than 5 min. Over-incubating causes genomic DNA contamination and plasmid damage*.

4. **Add 2 ml Buffer A3, mix immediately but thoroughly by inverting 5-6 times.** *It is important to mix the solution well, if the mixture still appears conglobated, more mix is required*.

5. Two options for clearing the lysates:

   a. High speed centrifuge**: Transfer the lysate to a high speed centrifuge tube and centrifuge at 13,000 rpm (14,000-18,000 g) for 50 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube.**

   b. EasyFilter syringe: **place the EasyFilter syringe to a clean 50 ml conical tube with the plunger removed. Pour the lysate into the filter syringe barrel. Allow the lysate to sit for 5-10 min. The white precipitates should float to the top. Hold the filter syringe barrel over the 50 ml tube and gently insert the plunger to expel the cleared lysate to the tube. Stop when feel resistance. some of the lysate may remain in the flocculent precipitate.**

6. **Add 4 ml Buffer A3 and 5 ml Ethanol (96-100%). Mix well by inverting 5-6 times.** *Proceed with either centrifuge or vacuum method described on Page 7 or 9*.

Trouble shooting guide

Low Yield	Poor Cell lysis.	Resuspend the pellet well prior adding buffer A2. Mix the lysate well after adding A2. Make fresh buffer A2 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 A2.	Do not vortex or mix aggressively after adding Buffer A2. Do not incubate more than 5 min after adding Buffer A2.
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.

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