# EasyPrep™ EndoFree Plasmid Midiprep Manual

Catalog#: PD03-21, PD03-22, PD03-23

PD03-31, PD03-32, PD03-33


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**If precipitate form in buffers, warm at 37 °C to dissolve before use. For research use only**


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## Table of Contents



## Trouble Shooting Guide

| Problems | Possible Reasons | Suggested Improvements |
|---|---|---|
| Low Yield | Poor Cell lysis. | Resuspend pellet thoroughly by votexing and pipetting prior adding Buffer A2.<br><br>Order fresh Buffer A2 if the cap had not been closed tightly. |
| | Bacterial culture overgrown or not fresh. | Grow bacterial 12-16 hours. Spin down cultures and store the pellet at - 20°C if the culture is not purified the same day. Do not store culture at 4°C overnight. |
| | Low copy-number plasmid. | Increase culture volume according to instructions on page 9. |
| No DNA | Plasmid lost in Host *E.coli* | Prepare fresh culture. |
| 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 minutes after adding Buffer A2. |
| 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. |
| No phase partitioning after centrifugation | Temperature is lower than 23°C. | Make sure the temperature is greater than 23°C for centrifugation or incubate the sample at 60°C for 5 min and then perform centrifugation |

## Purification of low-copy plasmid DNA and Cosmids

The Midiprep protocols can be used for preparation of low-copy-number plasmid DNA or cosmids from 200 ml overnight E. coli cultures grown in LB medium.

The following slight modifications to the protocols are required:

- Doubling the volumes of Buffers A1, A2, N3 and RET.
- When plasmid DNA or cosmids are >10 kb, preheat Buffer EB (or water) to 70°C prior to eluting DNA.
- A 200 ml overnight LB culture typically yields 100–200 µg DNA.

## 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.*



## Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient reversible binding of DNA to the mini column while proteins and other impurities are removed by wash buffer. Nucleic acids are then eluted with sterile water or elution buffer.

Plasmid isolated with traditional protocol normally contains high level of endotoxins (lipopolysaccharides or LPS). For transfection of endotoxin sensitive cell lines or microinjection, the endotoxins should be removed before the applications. The EasyPrep™ endoFree system uses a specially formulated buffer that extracts the endotoxin from the plasmid DNA. Two rounds of extraction will reduce the endotoxin level to 0.1 EU (Endotoxin) per µg of plasmid DNA. The endoFree plasmid miniprep kit provides an efficient endotoxin removal step into the traditional purification procedure to produce transfection grade plasmid DNA.

This kit is designed for fast and efficient purification of plasmid DNA from 3 to 10 ml of *E. coli* culture. The mini column II has a DNA binding capacity of 80 µg. The purified endoFree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

## Important Notes

**Plasmid 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 2 times. Please reference Table 1 for the commonly used plasmids.

**Table 1. Commonly used plasmid and expected yield.**

| Plasmid | Origin | Copy Num-bers | Expected Yield (µg/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 |
| pGEMR | Muted pMB1 | 300-400 | 6-7 |
| pBluescriptR | 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 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. Please reference Table 2 for the *endA* information.

| ***EndA*$^-$ Strains of *E. Coli*** | | | | | | | |
|---|---|---|---|---|---|---|---|
| DH5α | DH1 | DH21 | JM106 | JM109 | SK2267 | SRB | XLO |
| TOP10 | DH10 B | JM10 3 | JM107 | SK1590 | MM294 | Stbl2™ | XL1-Blue |
| BJ5182 | DH20 | JM10 5 | JM108 | SK1592 | Se-lect96™ | Stbl4™ | XL10-Gold |
| ***EndA*$^+$ Strains of *E. Coli*** | | | | | | | |
| C600 | JM110 | RR1 | ABLE® C | CJ236 | KW251 | P2392 | BL21(DE3) |
| HB101 | TG1 | TB1 | ABLE® K | DH12S™ | LE392 | PR700 | BL21(DE3) pLysS |
| JM101 | JM83 | TKB1 | HMS174 | ES1301 | M1061 | Q358 | BMH 71-18 |
| All NM strains | | | | All Y strains | | | |

**Optimal Cell Mass ($OD_{600}$ x ml of Culture):** This procedure is designed for isolating plasmid grown in standard LB medium 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 mini column II has an optimal biomass of 10-36. For example, if the $OD_{600}$ is 3.0, the optimal culture volume should be 3-12 ml.

**Culture Volume:**

1. Use a flask or a culture tube 4 times bigger in volume than the culture medium to secure optimal condition for bacteria growth. *For example, 14ml culture tube can be used to culture up to 3.5ml LB.*
2. Don't exceed the maximum culture volume suggested in the protocol. Incomplete lysis due to over amount of bacterial culture results in lower yield and less purity.



## EndoFree Plasmid Midiprep Protocol

(Vacuum Method, preferred)

1. Set up the vacuum manifold according to manufacture's instruction and connect the column to the manifold.
2. Carry out step 1-8 in previous protocol on page 8.
3. Carefully transfer **3.5 ml** lysate mixture from step 8 in the previous protocol to a DNA column and turn on the vacuum to allow the lysate pass through the column. Repeat until the remaining solution pass through the column.
4. Add **3.5 ml Buffer KB** to the column and allow the buffer pass the column by vacuum.
5. Add **3.5 ml DNA Wash Buffer** to the column and allow the vacuum to draw the liquid through the manifold. Turn off the vacuum. Repeat once.
6. Transfer the column to a 15 ml conical tube and centrifuge at 3500 rpm for 15 minute to remove the residual ethanol.
7. Transfer the column to an new endoFree sterile 15 ml conical tube and add **0.5 ml Elution Buffer**. Incubate for 1 minute and centrifuge at 3500 rpm for 5 minute to elute DNA. Reload the eluate into the column (use the same 15 ml tube) and incubate for 1 minute, centrifuge at 3500 rpm for 5 minute to elute DNA. *The eluted DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.*

**insert the plunger to expel the clear lysate.**

7. Column preparation: during centrifuge, add **1 ml Column Buffer** to the column, incubate for 2 min, centrifuge at 3500 rpm for 5 min. *This ensures the column having optimal binding capacity. Especially when the kit is stored over 6 month or the yield declines noticeably.*
8. Carefully transfer about **4 ml clear lysate** (no more than 800 µl) to a clean 15 ml tube and add **4 ml Buffer RET.** Mix well by sharp hand shaking for 3 times.
9. Transfer **3.5 ml lysate mixture** to a DNA column and centrifuge at 3500 rpm for 5 min. Discard the flow-through liquid. Repeat the procedure till all the lysate mixture gone though the column.
10. Add **2.5 ml Buffer KB** into the spin column, centrifuge at 3500 rpm for 5 min. Remove the spin column from the tube and discard the flow-through. Put the column back to the collection tube.
11. Add **3.5 ml DNA Wash Buffer** and centrifuge at 3500 rpm for 5 min. Discard the flow-through liquid and insert the column back to the collection tube. Repeat once.
12. Centrifuge the column at 3500 rpm for 15 minute to remove the residual ethanol. *It is critical to remove residual ethanol for optimal elution. Residual ethanol may interfere subsequent applications.*
13. Transfer the column to a new sterile 1.5 ml tube and add **0.5 ml Elution Buffer** (TE buffer, pH8.5). Incubate for 1 minute at room temperature and centrifuge at 3500 rpm for 5 min to elute DNA. Reload the eluate into the column (use the same 15 ml tube) and incubate for 1 minute, centrifuge at 3500 rpm for 5 min to elute DNA. *Two elutions give rise to maximum DNA yield. Use less Elution Buffer if high concentration is desired. The eluted DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.*



## 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 (22-25°C). The guaranteed shelf life is 12 months from the date of purchase.

## Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps and pay special attention to the followings,

## Important

- RNase A: It is stable for 6 month under room temperature. Spin down the vial briefly, add the RNase A solution to buffer A1 and mix well before use. Keep Buffer A1 in 4°C refrigerator since.
- Add 96-100% ethanol to each DNA Wash Buffer bottle before use.
- **Buffer A2 precipitates below room temperature.** It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.
- **Buffer N3 may form precipitates below 10°C,** warm up at 37°C to dissolve the precipitates before use.
- **Keep the cap tightly closed for Buffer A2 after use.**

***Carry out all centrifugations at room temperature.***

## Materials supplied by users

- 96-100% ethanol
- 1.5 ml and 2.0 ml microcentrifuge tubes.
- High speed microcentrifuge or Vacuum manifold.

# Kit Contents

| Catalog # | PD03-21<br>PD03-31 | PD03-22<br>PD03-32 | PD03-23<br>PD03-33 |
|---|---|---|---|
| DNA Columns | 10 | 25 | 50 |
| EasyFilter | 10<br>(PD03-31) | 25<br>(PD03-32) | 50<br>(PD03-33) |
| Buffer A1 | 30 ml | 70 ml | 140 ml |
| Buffer A2 | 30 ml | 70 ml | 140 ml |
| Buffer N3 | 15ml | 70 ml | 140 ml |
| Buffer KB | 30 ml | 70 ml | 140 ml |
| Buffer RET | 50 ml | 125 ml | 250ml |
| DNA Wash Buffer * | 20 ml | 50 ml | 100 ml |
| Elution Buffer | 10 ml | 25 ml | 50 ml |
| Column Buffer | 12 ml | 30 ml | 60 ml |
| RNase A | 110 µl | 240 µl | 480 µl |
| User Manual | 1 | 1 | 1 |

*DNA Wash Buffer is a 5x Concentrate and needs to be diluted with ethanol before use: add 80 ml (PD03-21, PD03-31) or 200ml (PD03-22, PD03-32) 96-100% ethanol to each DNA Wash Buffer bottle before use.

## Safety Information

- Buffer N3 contain acetic acid, wear gloves and protective eyewear when handling.
- Buffer N3, KB and RET contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.



## EndoFree Plasmid Midiprep Protocol

(Centrifuge Method)

1. Inoculate **50 ml** LB containing appropriate antibiotic with a single colony from a freshly streaked selective plate. Grow at 37°C for 14-16 hours with vigorous shaking.
   - *Do not use a streaked plate that has been stored at 4°C.*
   - *Do not inoculate culture directly with glycerol stock.*
   - *This protocol is optimized for E. coli strain cultured in LB medium. When using TB or 2xYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD600). Buffers need to be scaled up if over amount of cultures are being processed.*

2. Harvest the bacterial culture by centrifugation at 13,000 rpm for 3 minute. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely. *Residual medium will cause poor cell lysis and thus lower DNA yield, loose pellet after centrifugation in step 6.*

3. Add **2.5 ml Buffer A1** and completely resuspend bacterial pellet by vortexing or pipetting. Complete resuspension is critical for bacterial lysis and lysate neutralization.

4. Add **2.5 ml Buffer A2**, mix gently by inverting 5-6 times (do not vortex) and incubate at room temperature for 5 minutes. *Do not incubate for more than 5 minutes, which will increase the possibility of genomic DNA contamination.*

5. Add **1.25 ml Buffer N3**, mix completely by inverting the tube for 5-6 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. 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. EasyFilter syringe: **Spin the sample at 3,500 rpm for 5 min at room temperature. Place the EasyFilter syringe to a 15ml conical 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**