*Ver: 2405*

# Ezgene™ EndoFree Plasmid Miniprep Kit

# （BW-PD1212）

## Contents

## Kit Contents

| Catalog# | BW-PD1212-00 | BW-PD1212-01 | BW-PD1212-02 |
|---|---|---|---|
| Preps | 10 | 50 | 250 |
| Mini Columns | 10 | 50 | 250 |
| 2 mL Collection Tubes | 10 | 50 | 250 |
| Buffer GBL | 8 mL | 30 mL | 150 mL |
| Buffer A1 | 5 mL | 25 mL | 115 mL |
| Buffer B1 | 5 mL | 25 mL | 115 mL |
| Buffer N3 | 6 mL | 30 mL | 160 mL |
| Buffer KB | 6 mL | 30 mL | 130 mL |
| DNA Wash Buffer* | 3 mL | 15 mL | 3 x 24 mL |
| EndoClean Buffer | 2 mL | 10 mL | 40 mL |
| Endofree Elution Buffer | 2 mL | 10 mL | 40 mL |
| RNase A (20 mg/mL) | 25 µL | 125 µL | 575 µL |
| User Manual | 1 | 1 | 1 |

*Add 12 mL (BW-PD1212-00) or 60 mL (BW-PD1212-01) or 96 mL (BW-PD1212-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

## Introduction

Key to this kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily 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 Ezgene™ 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 12 mL of *E.coli* culture. The Mini Column has a plasmid 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.

## Storage and Stability

Buffer A1 should be stored at 4℃ once RNase A is added. All other materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 12 months from the date of production.

## Before Starting

Alternative endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA, while Protocol B removes endotoxin after the purification of plasmid DNA.

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

## Important Notes

✪ RNase A: It is stable for one year under room temperature. Spin down RNase A vial briefly. Add the RNase A to Buffer A1 and mix well before use.

✪ Add 12 mL (BW-PD1212-00) or 60 mL (BW-PD1212-01) or 96 mL (BW-PD1212-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

✪ 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 12,000 rpm.
- *Carry out all centrifugations at room temperature.*

## Materials supplied by users

- 96- 100% ethanol.
- 1.5 mL and 2.0 mL pyrogen free microcentrifuge tubes.
- 15 mL tube.
- High speed microcentrifuge.
- Vacuum manifold if vacuum protocol is applied.

## Safety Information

Buffer N3 contains acidic acid, wear gloves and protective eyewear when handling.

Buffer N3 and KB contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

# EZgene ™ EndoFree Plasmid Miniprep Protocol

## A: Removal of Endotoxin during Plasmid Purification

This protocol is designed for removing the endotoxin during the plasmid purification.

**A1. Spin Protocol**

1. Inoculate ***3-12 mL*** LB containing appropriate antibiotic with a fresh colony. Grow at 37℃ for 14-16 hours with vigorous shaking.

   **Note:** This protocol is optimized for *E. coli* strain cultured in LB medium. When using TB or 2 × YT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 ($OD_{600}$). Buffers need to be scaled up proportionally if over amount of cultures are being processed.

   **Note:** Do not use a starter culture that has been stored at 4℃.

   **Note:** Do not grow starter culture directly from glycerol stock.

   **Note:** The culture can be centrifuged at 6,000 rpm in a 15 mL conical tube for 10 minutes if high speed centrifuge tubes are not available. Alternatively, the cultures can also be spin down in multiple 2 mL tubes.

2. Column equilibration: Place a Mini Column in a clean collection tube, and add **500µL Buffer GBL** to Mini Column. Centrifuge for 1 min at 12,000 rpm (~13,400 × g) in a table-top centrifuge. Discard the flow-through, and set the Mini Column back into the collection tube. (Please use freshly treated spin column).

3. Harvest bacterial culture by centrifugation for 1 minute at 12,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

   **Note:** Residue medium will cause poor cell lysis and thus lower DNA yield.

4. Add **450 µL Buffer A1** (*Add RNase A to Buffer A1 before use*) and completely resuspend bacterial pellet by vortexing or pipetting**.**

   **Note:** Complete resuspension is critical for bacterial lysis and lysate neutralization.

5. Add **450 µL Buffer B1**, mix gently by inverting 10 times (*do not vortex*) and incubate at room temperature for 5 minutes.

   **Note:** Do not incubate for more than 5 minutes.

   **Note:** Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 37℃ to dissolve the precipitations before use.

6. Add **100 µL Buffer N3**, mix completely by inverting the vial for 5-10 times.

   **Note:** 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.

7. Centrifuge the lysate at 12,000 rpm for 10 minutes at room temperature.

   **Note:** If the lysate doesn't appear clean, reverse the tube angle, centrifuge for 5 more minutes.

8. Carefully transfer the clear lysate into **2 mL Collection Tube** and add **0.1 volume** of **EndoClean Buffer.** Mix by vortexing till homogeneous and incubate on ice for 10 minutes. Mix by tapping the tube several times during incubation.

   **Note:** The solution becomes red and turbid after adding EndoClean Buffer. The solution becomes clear after incubation on ice.

   **Note:** Use a serological pipet or a tip cut with a clean razor in the end to transfer the EndoClean Buffer.

   **Note:** Mix the sample several times during incubation without leaving ice.

9. Centrifuge the solution at 12,000 rpm for 10 minutes at room temperature (the temperature must be greater than 23℃). The solution should separate into 2 phases, the upper clear phase contains DNA and the lower organic phase contains endotoxin. The two phases will not separate if the temperature is less than 23℃.

   **Note:** If phase partitioning is not observed after centrifugation: incubate the solution at 65℃ for 5 minutes. The solution becomes turbid again. And then repeat step **8**. Or add 200 µL Chloroform (37℃), vortex to mix well, repeat step **8**.

   **Note:** Up to 99% of the endotoxin can be removed by extracting with the EndoClean Buffer once. Another extraction is necessary if less than 0.1 EU (Endotoxin)/ µg of DNA is desired by repeating step **7-8**.

10. Transfer the clear phase lysate, avoid the colored phase, to a clean 2 mL tube. Add **450 µL Buffer N3** and **400 µL 100% ethanol** to the tube. Mix well by sharp hand shaking for 3 times.

11. Transfer **700 µL** of the lysate/ethanol mixture to the **pretreated Mini Column** and centrifuge at 12,000 rpm for 30 s. Decant the flow-through liquid and insert the **Mini Column** back to the collection tube. Transfer the remaining solution to the **Mini Column** and centrifuge at 12,000 rpm for 30 s. Decant the flow-through liquid and insert the **Mini Column** back to the collection tube.

12. Add **500 µL Buffer KB** into the **Mini Column**, centrifuge at 12,000 rpm for 1 minute. Remove the **Mini Column** from the tube and discard the flow-through. Put the **Mini Column** back to the collection tube.

    **Note:** This step is important to remove residual protein contaminations especially for *EndA+* strains and be highly recommended for high quality plasmid DNA.

13. Add **600 µL** of **DNA Wash Buffer** and centrifuge at 12,000 rpm for 30 s. Decant the flow-through liquid and insert the **Mini Column** back to the collection tube. Repeat step **13**.
14. Reinsert the **Mini Column** back to the collection tube, **with the lid open**, Centrifuge at 12,000 rpm for 5-10 minutes.

    **Note:** It is critical to removes ethanol residues completely. The remaining ethanol will inhibit the elution of DNA from the column.
15. Transfer the **Mini Column** to an endofree 1.5 mL tube and add **100-150 µL** of **Endofree Elution Buffer**（**Supplied**）. Incubate for 1 minute and centrifuge at 12,000 rpm for 1 minute to elute DNA. Reload the eluate into the **Mini Column** (use the same 1.5 mL tube) and incubate for 1 minute, centrifuge at 12,000 rpm for 1 minute to elute DNA.

    **Note:** The DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.
16. Determination of DNA concentration,

    **Concentration (µg/mL) =$OD_{260}$×50×dilution factor.**

    **Note:** Two elution give rise to maximum DNA yield. Use less EndoFree Elution Buffer if high concentration is desired.

**A2. Spin/Vacuum Protocol**

Set up the vacuum manifold according to manufacture's instruction and connect the column to the manifold.

Carry out step **1-10** in previous protocol on page **5-6**.

1. Carefully transfer the clear lysate from step **10** in the previous protocol to **pretreated Mini Column** and turn on the vacuum to allow the lysate pass through the column.
2. Add **500 µL Buffer KB** into the **Mini Column** and allow the buffer pass the column by vacuum.

   **Note:** This step is important to remove residual protein contaminations especially for *EndA+* strains and be recommended for high quality plasmid DNA.
3. Add **600 µL** of **DNA Wash Buffer** to the **Mini Column** and allow the vacuum to draw the liquid through the manifold. Turn off the vacuum. Repeat step **3**.
4. Transfer the **Mini Column**, with the lid open, to a **2 mL Collection Tube** and centrifuge at 12,000 rpm for 2 minutes.
5. Transfer the **Mini Column** to an endofree 1.5 mL tube and add **100-150 µL** of **Endofree Elution Buffer**（Supplied）. Incubate for 1 minute and centrifuge at 12,000 rpm for 1 minute to elute DNA. Reload the eluate into the **Mini Column** (use the same 1.5 mL tube) and incubate for 1 minute, centrifuge at 12,000 rpm for 1 minute to elute DNA.

   **Note:** The DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

# EZgene ™ EndoFree Plasmid Miniprep Protocol

## B: Removal of Endotoxin after Plasmid Purification

This protocol is designed for removing the endotoxin after the plasmid is purified.

1. Follow the protocol on page **5-6** from step **1** to **7**.
2. Carefully transfer the clear lysate to a 1.5 mL tube and add **500 µL Buffer N3** and **400 µL 100% ethanol**. Mix well by sharp hand shaking for 2 times and go to step **11-15** on page **6-7**.

   **Note:** The plasmid DNA is purified. The following steps are for removal of endotoxin.
3. After the plasmid is purified, add **0.1 volume** of **EndoClean Buffer** to the plasmid sample in a 2 mL centrifuge tube (For example, add **10 µL EndoClean Buffer** to 100 µL plasmid sample). The solution becomes turbid after adding **EndoClean Buffer**. If the temperature is below 23℃, the solution remains clear.
4. Vortex the sample for 10 s and incubate the tube on ice for 10 minutes. Mix the sample several times without leaving ice. The solution becomes clear after incubating on ice.
5. Centrifuge at 12,000 rpm at room temperature for 10 minutes (the temperature must be greater than 23℃ for phase partitioning). Carefully transfer the upper clear layer solution to another 2 mL tube.

   **Note:** If phase partitioning is not observed after centrifugation, add 200 µL Chloroform, vortex for 10 s, and repeat step **5**.
6. Precipitate plasmid DNA with 0.1 volume of 3 M KAc (pH 5.2) and 0.7 volume of Isopropanol. Centrifuge at 12,000 rpm for 10 minutes. Carefully decant the supernatant.
7. Add 1 mL 70% ethanol and centrifuge at 12,000 rpm for 5 minutes.
8. Carefully decant and air-dry the DNA for 30 minutes in a hood. Resuspend the DNA with **Endofree Elution Buffer**（Supplied）.

   **Note:** The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, in vitro translation, sequencing, transfection, and microinjection.

## Purification of Low-Copy-Number Plasmid/Cosmid

The yield of low copy number plasmid is normally around 0.1–1 µg/mL of overnight culture. For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

✪ Culture volume: Use **2×volumes** of the high copy number culture. Use up to 25 mL for miniprep.

✪ Use **2×volumes** of the **Buffer A1, Buffer B1, Buffer N3,** and **Buffer KB**. Additional buffers can be purchased from Biomiga.

✪ Use **same volume** of **DNA Wash Buffer** and **Endofree Elution Buffer**.

## Trouble Shooting Guide

| Problems | Possible Reasons | Suggested Improvements |
|---|---|---|
| Low yield | Poor cell lysis. | Resuspend pellet thoroughly by vortexing and pipetting prior adding Buffer B1. Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2 M 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 -20℃ if the culture is not purified the same day. Do not store culture at 4℃ overnight. |
| Low yield | Low copy-number plasmid. | Increase culture volume according to instructions on page 10. |
| 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 Buffer 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 were 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 BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO 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 or visit our website.

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