***Ver: 1904***

**Endofree Express Plasmid Miniprep Kit**

**（BW-PD1219）**

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# Kit Contents

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| **Catalog #** | **BW-PD1219-00** | **BW-PD1219-01** | **BW-PD1219-02** |
| Preps | 10 | 50 | 250 |
| Mini Columns (White ring) | 10 | 50 | 250 |
| Lysate Clearance Columns (Green ring) | 10 | 50 | 250 |
| 2 mL Collection Tubes | 10 | 50 | 250 |
| Buffer GBL | 8 mL | 30 mL | 150 mL |
| Buffer F1 | 2.4 mL | 12 mL | 60 mL |
| Buffer F2 | 2.4 mL | 12 mL | 60 mL |
| Buffer F3 | 2.4 mL | 12 mL | 60 mL |
| Buffer RET | 2.4 mL | 12 mL | 60 mL |
| Buffer KB | 6 mL | 30 mL | 150 mL |
| DNA Wash Buffer\* | 3 mL | 7 mL | 35 mL |
| Endofree Elution Buffer  | 1 mL | 7 mL | 35 mL |
| RNase A (20 mg/mL) | 12 µL | 60 µL | 300 µL |
| User Manual | 1 | 1 | 1 |

\*Add 12 mL (BW-PD1219-00) or 28 mL (BW-PD1219-01) or 140 mL (BW-PD1219-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

# Introduction

Key to the kit is our proprietary Lysate Clearance Column that allows the clearance of lysate from 1-2 mL *E. coli* culture in 30 seconds. The whole procedure takes only 10 minutes. There are no chaotropic salts in the buffer system. It is the only eco-friendly glass fiber based plasmid miniprep kit on the market.

This kit uses a specially formulated buffer that removes endotoxin from the plasmid DNA. The purified DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

# Storage and Stability

Buffer F1 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

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

◍Buffer F1: Spin down the RNase A vial and add to Buffer F1, store Buffer F1/RNase A at 4℃.

◍Buffer F2: Warm up at 37℃ to dissolve if precipitation forms.

◍Add 96-100% ethanol to DNA Wash Buffer before use.

# Materials not Supplied

❂High speed microcentrifuge or vacuum manifold.

❂96-100% ethanol.

❂1.5 mL tubes.

# Safety Information

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

# Protocol

1. 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).
2. Inoculate ***1-2 mL*** of fresh bacterial culture by centrifugation for 1 min at 12,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium.
3. Add **200 μL Buffer F1** (*Add* ***RNase A*** *to Buffer F1 before use*) and completely resuspend bacterial pellet by vortexing or pipetting.

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

1. Add **200 μL Buffer F2**, mix gently by inverting the tube 10 times (*do not vortex*), and incubate at room temperature for 1-2 min until the solution becomes clear.
2. Add **200 μL Buffer F3** to the sample from step **3**, mix completely by inverting/sharp shaking the vial for 5 times and incubate at room temperature for 2 min.
3. Transfer the whole lysate to a **pretreated Lysate Clearance Column (Green ring)**. Centrifuge at 10,000 rpm for 30 s.

**Note:** If the lysate still remains in the column, spin for another 30 s.

1. Discard the **Lysate Clearance Column** and add **200 µL Buffer RET** and 200 µL 100% ethanol to the flow through in thecollection tube, mix well by pipetting.
2. Transfer **750 µL** cleared lysate to the **pretreated Mini Column (White ring)** with a **2 mL Collection Tube**. Spin at 12,000 rpm for 30 s. Discard the flow through and put the **Mini Column** back to the collection tube. Process the remaining sample as described.
3. Add **500 μL Buffer KB**, centrifuge at 12,000 rpm for 30 s. Discard the flow-through and put the **Mini Column** back to the collection tube.

**Note:** This step is NOT necessary if the plasmid is being purified from *endA-* strain such as DH5α and Top10.Buffer KB is necessary for *endA+* strains such as HB101, JM110, JM 101 and their derived strains.

1. Add **600 μL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **Mini Column**, centrifuge at 12,000 rpm for 30 s and discard the flow-through.

Repeat step **10**.

1. Reinsert the **Mini Column** into the collection tube and centrifuge for 1 minute at 12,000 rpm.

**Note:** Residual ethanol can be removed more efficiently with the column lid open.

1. Carefully transfer the **Mini Column** into a clean 1.5 mLtube and add **50-100 μL** **Endofree** **Elution Buffer** into the center of the column and let it stand for 1 minute. Elute the DNA by centrifugation at 12,000 rpm for 30 s.

**Optional:** Reload the eluate into the center of the column for a second elution.

**Note:** The first elution normally yields around 70% of the plasmid DNA. Add the eluted DNA back to the column for another elution yields 20-30% of the DNA.

1. The DNA concentration can be calculated as follows,

**Concentration (μg/mL)=OD260×50×dilution factor.**

# Trouble Shooting Guide

|  |  |  |
| --- | --- | --- |
| **Problems** | **Possible reasons**Wash and dry | **Solutions** |
| Low DNA yield | 1. Poor cell lysis.
2. Bacterial culture overgrown or not fresh.
 | 1. Make fresh Buffer F2 if the cap had not been closed tightly.
2. 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.
 |
| No DNA yield | Plasmid lost in host *E.coli.* | Prepare fresh culture. |
| Genomic DNA contamination | Over-time incubation after adding Buffer F2. | Do not vortex or mix aggressively after adding Buffer F2. Do not incubate more than 5 minutes after adding Buffer F2. |
| RNA contamination | RNase A not added to Buffer F1. | Add RNase A to Buffer F1. |
| Plasmid DNA floats out of wells while running in agarose gel | Ethanol traces were not completely removed from column. | Make sure that no ethanol residual remaining in the silicon membrane before eluting 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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