Ver: 1904

Express Plasmid Miniprep Kit (10 minutes)

（BW-PD1218）

Contents

Kit Contents

Catalog #	BW-PD1218-00	BW-PD1218-01	BW-PD1218-02	BW-PD1218-03
Preps	10	50	100	250
Mini Columns (White ring)	10	50	100	250
Lysate Clearance Columns (Green ring)	10	50	100	250
2 mL Collection Tubes	10	50	100	250
Buffer GBL	8 mL	30 mL	60 mL	150 mL
Buffer F1	2.4 mL	12 mL	24 mL	60 mL
Buffer F2	2.4 mL	12 mL	24 mL	60 mL
Buffer F3	2.4 mL	12 mL	24 mL	60 mL
Buffer KB	6 mL	30 mL	60 mL	150 mL
DNA Wash Buffer*	3 mL	7 mL	15 mL	35 mL
RNase A (20 mg/mL)	12 µL	60 µL	120 µL	300 µL
Elution Buffer	1 mL	7 mL	14 mL	30 mL
User Manual	1	1	1	1

*Add 12 mL (BW-PD1218-00) or 28 mL (BW-PD1218-01) or 60 mL (BW-PD1218-02) or 140 mL (BW-PD1218-03) 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 mL of *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.

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.

Important Notes

- RNase A: Spin down the RNase A vial briefly. Add the RNase A to Buffer F1 and mix well before use.
- Buffer F2 may precipitates below room temperature. It is critical to warm up at 37℃ to dissolve the precipitates before use. Keep the cap tightly closed for Buffer F2 after use.
- Add 12 mL (BW-PD1218-00) or 28 mL (BW-PD1218-01) or 60 mL (BW-PD1218-02) or 140 mL (BW-PD1218-03) 96-100% ethanol to each DNA Wash Buffer bottle before use.
- Carry out all centrifugations at room temperature. Ensure the availability of centrifuge capable of 12,000 rpm.

Materials not Supplied

- 96-100% ethanol
- High speed centrifuge
- 1.5 mL tubes

Safety Information

Buffer F3 contains acidic acid, wear gloves and protective eyewear when handling.

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. Harvest ***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. Remove the residue medium completely.

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.

4. Add **200 μL Buffer F2**, mix gently by inverting 10 times (*do not vortex*), and incubate at room temperature for 1-2 minutes until the solution becomes clear.

5. Add **200 μL Buffer F3** to the sample from step **3**, mix completely by inverting the vial for 5 times and incubate at room temperature for 2 min.

6. Transfer the whole lysate to the **Lysate Clearance Column (Green ring)**. Centrifuge at 10,000 rpm for 30 s.
   Note: If the lysate remains in the column, spin for another 30 s.

7. Discard the **Lysate Clearance Column** and add 200 μL 100% ethanol to the flow through in the collection tube, mix well by pipetting.

8. Transfer **750 μL** cleared lysate to the **pretreated Mini Column (White ring)** with a **2 mL Collection Tube** and spin at 12,000 rpm for 30 s. Discard the flow through and put the column back to the collection tube.

9. Add **500 μL Buffer KB** and 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.

10. 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**.

11. Reinsert the **Mini Column** into the **2 mL Collection Tube** and centrifuge for 1 min at 12,000 rpm.

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

12. Carefully transfer the **Mini Column** into a clean 1.5 mL tube and add **50-100 µL Elution Buffer** into the center of the column. Let it stand for 1 min. 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.

13. The DNA concentration can be calculated as follows,

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

Trouble Shooting Guide

Problem	Possible Reason	Suggested Improvement
Low Yield	Poor cell lysis.	Resuspend pellet thoroughly by vortexing and pipeting prior adding Buffer F2. Make fresh Buffer F2 if the cap had not been closed tightly (Buffer F2: 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.	Scale up the volume of buffers accordingly.
No DNA	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, 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 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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