Ver: 1904

All Inclusive Plasmid Miniprep Kit

（BW-PD1228）

Contents

Kit Contents

Catalog#	BW-PD1228-00	BW-PD1228-01	BW-PD1228-02
Preps	10	50	250
Mini Columns (White ring)	10	50	250
Lysate Clearance Columns (Green ring)	2	10	50
2 mL Collection Tubes	10	50	250
Buffer GBL	8 mL	30 mL	150 mL
Buffer A1	5 mL	27 mL	135 mL
Buffer B1	5 mL	27 mL	135 mL
Buffer N1	6 mL	33 mL	165 mL
Buffer N3	3 mL	12 mL	60 mL
Buffer KB	6 mL	30 mL	150 mL
Buffer RET	1 mL	6 mL	30 mL
DNA Wash Buffer*	3 mL	15 mL	3×24 mL
Endofree Elution Buffer	2 mL	9 mL	45 mL
RNase A (20 mg/mL)	25 µL	135 µL	675 µL
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*Add 12 mL (BW-PD1228-00) or 60 mL (BW-PD1228-01) or 96 mL (BW-PD1228-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

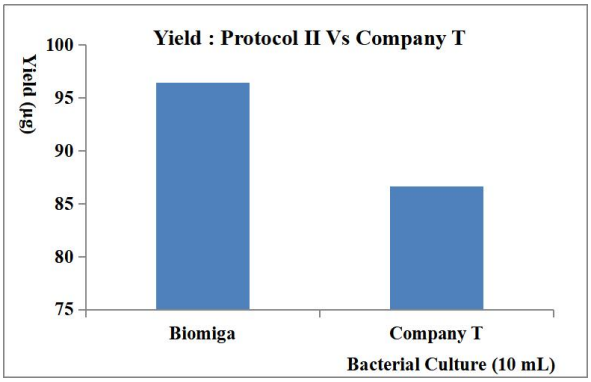
Introduction

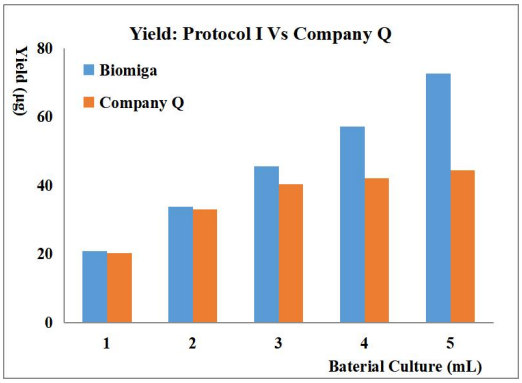
This kit is designed to purify plasmid from 1-12 mL of culture for all possible downstream applications. You can select one of the four protocols listed accordingly.

Here are the key points,

- Highest yield: Up to 120 µg per prep (Protocol II)
- Fast processing time: 10 minutes (Protocol III)
- Highest purity: Transfection grade (Protocol IV)
- Eco-friendly: No chaotropics (Protocol III)
- Safe for researchers: No chaotropics (Protocol III)

Options	Mini (Protocol I)	Mini II (Protocol II)	Express Mini (Protocol III)	Endofree Express Mini (Protocol IV)
Culture volume	1 - 5 mL	1-12 mL	1-2 mL	1-5 mL
Lysate clearance	Centrifugation	Centrifugation	Lysate Clearance Column	Centrifugation
Elution volume	50 µL	100 µL	50 µL	50 µL
Yield (high copy number plasmid)	up to 50 µg	up to 120 µg	up to 30 µg	up to 50 µg
Preparation time	< 25 min	< 30 min	< 10 min	< 30 min
Purity	Molecular Biology Grade	Molecular Biology Grade	Molecular Biology Grade	Transfection Grade
Processing	Centrifuge, Vacuum	Centrifuge, Vacuum	Centrifuge, Vacuum	Centrifuge, Vacuum
Application	Fluorescent and radioactive sequencing (including capillary sequencing), ligation, cloning, transformation, etc.	Fluorescent and radioactive sequencing (including capillary sequencing), ligation, cloning, transformation, etc.	Fluorescent and radioactive sequencing (including capillary sequencing), ligation, cloning, transformation, etc.	Fluorescent and radioactive sequencing (including capillary sequencing), ligation, cloning, transformation, an d transfection, etc.





Storage and Stability

All materials can be stored at room temperature (15-25℃). The guaranteed shelf life is 12 months from the date of production.

Before Starting

Familiar with each step by reading this manual and prepare all materials for the procedure.

Important Notes

- RNase A: 20 mg/mL. It is stable for one year at room temperature. Spin down the RNase A vial briefly. Add the RNase A to Buffer A1 and mix well before use. Store Buffer A1/RNase A mixture at 4℃.
- Buffer A1: Spin down the RNase A vial and add to Buffer A1, mix well before use.
- Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 37℃ to dissolve the precipitates before use. Keep the cap tightly closed for Buffer B1 after use.
- Add 96-100% ethanol to DNA Wash Buffer before use.
- Ensure the availability of centrifuge capable of 12,000 rpm.
- *Carry out all centrifugations at room temperature.*

Materials not Supplied

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

Safety Information

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

Buffer KB and N1 contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

Protocol I: Processing 1-5 mL Culture

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 ***1-5 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 **250 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.

4. Add **250 μL Buffer B1**, mix gently by inverting 10 times (*do not vortex*), and incubate at room temperature for 5 minutes until the solution becomes clear.
   Note: Do not incubate for more than 5 minutes.

5. Add **350 μL Buffer N1** to the sample from step **3**, mix completely by inverting/shaking the vial for 5 times. Centrifuge at 12,000 rpm for 10 min.
   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.

6. Carefully transfer the clear lysate into the **pretreated Mini Column (White ring)** inserted in a **2 mL Collection Tube**, avoid the precipitations, spin at 12,000 rpm for 1 min, discard the flow-through and put the column back to the collection tube.
   Note: If the lysate doesn't appear clean, centrifuge for 5 more minutes and then transfer the clear lysate to Mini Column.

7. Add **500 μL Buffer KB** and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and put the 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.

8. Add **500 μL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **Mini Column**, centrifuge at 12,000 rpm for 1 minute and discard the flow-through.

Repeat step **8**.

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

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

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

    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.

11. The DNA concentration can be calculated as follows,

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

Protocol II: Processing 1-12 mL Culture

For ***1-4 mL*** culture, reduce the volume of **Buffer A1**, **B1**, **N1** to **250 µL**, **250 µL** and **350 µL**, respectively. And use the same volume of **DNA Wash Buffer** and **EndoFree Elution Buffer**.

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 ***5-12 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 **450 µL Buffer A1** (*Add **RNase A** to Buffer A1 before use*) and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields). Transfer the sample to a 2 mL microtube.

4. Add **450 µL Buffer B1**, mix by inverting 10 times (do not vortex) and incubate at room temperature for 5 min until the solution becomes clear.

   Note: Do not incubate for more than 5 minutes.

5. Add **550 µL Buffer N1** to the sample from step **3**, mix completely by inverting shaking 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.

6. Centrifuge at 12,000 rpm for 10 min.

7. Carefully transfer **700 µL** clear lysate into the **pretreated Mini Column (White ring)** with a **2 mL Collection Tube**, avoid the precipitations, spin at 12,000 rpm for 1 min, discard the flow-through and put the column back to the collection tube.

   Note: If the lysate doesn't appear clean, centrifuge for 5 more minutes and then transfer the clear lysate to Mini Column.

8. Carefully transfer the remaining clear lysate to the column, centrifuge at 12,000 rpm for 1 min and discard the flow-through in the collection tube. Put the column back to the collection tube.

9. Add **500 µL Buffer KB** and centrifuge at 12,000 rpm for 1 min. Discard the flow-through and put the 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 TOP 10. Buffer KB wash is necessary for endA$^+$ strains such as HB101, JM110, JM101 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 1 min and discard the flow-through.

    Repeat step **10**.

11. Reinsert the **Mini Column** into the collection tube and centrifuge for 2 min at 12,000 rpm.

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

12. Carefully transfer the **Mini Column** into a clean 1.5 mL tube and add **100-150 µL EndoFree Elution Buffer** into the column and let it stand for 1 min. Elute the DNA by centrifugation at 12,000 rpm for 1 min.

    Optional: Reload the eluate into the column and centrifuge again to improve the recovery.

    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.

Protocol III: Express Miniprep (1-2 mL Culture)

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 A1** (*Add **RNase A** to Buffer A1 before use*) and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).

4. Add **200 µL Buffer B1**, mix by inverting 10 times (**do not vortex**) and incubate at room temperature for 2 min until the solution becomes clear.
   Note: Do not incubate for more than 5 minutes.

5. Add **200 µL Buffer N3** to the sample from step **3**, mix completely by inverting shaking the vial for 5 times. Incubate at room temperature for 2 min. Transfer the whole lysate to a **Lysate Clearance Column (Green ring)**.

6. Centrifuge at 10,000 rpm for 30 s.
   Note: If the lysate still 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 and transfer **750 µL** cleared lysate to the **pretreated Mini Column (White ring)** with a **2 mL Collection Tube**.

8. Spin at 12,000 rpm for 30 s. Discard the flow through and use 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 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 wash is necessary for *endA*+strains such as *HB101*, *JM110*, *JM101* and their derived strains.

10. Add **600 µL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the column, centrifuge at 12,000 rpm for 30 s and discard the flow-through.
    Repeat step **10**.

11. Reinsert the spin column into the collection tube and centrifuge for 1 min at 12,000 rpm.

Note: Residual ethanol will be removed more effectively with the column lid open.

12. Carefully transfer the spin column into a clean 1.5 mL tube and add **50-100 µL EndoFree Elution Buffer** into the column and let it stand for 1 min. Elute the DNA by centrifugation at 12,000 rpm for 30 s.

Optional: Reload the eluate into the column and centrifuge again to improve the recovery.

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.

Note: For protocol III, this kit only provides some Lysate Clearance Columns, if you need more, it is recommended to purchase our Express Plasmid Miniprep Kit (BW-PD1218) separately.

Protocol IV: Endofree Miniprep (1-5 mL Culture)

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 ***1-5 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 **250 μL Buffer A1** (*Add **RNase A** to Buffer A1 before use*) and completely resuspend bacterial pellet by vortexing or pipetting. (Complete resuspension is critical for optimal yields).

4. Add **250 μL Buffer B1**, mix by inverting 10 times (**do not vortex**) and incubate at room temperature for 5 min until the solution becomes clear.
   Note: Do not incubate for more than 5 minutes.

5. Add **60 μL Buffer N3**, mix completely by inverting/shaking the vial for 5 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.

6. Centrifuge at 12,000 rpm for 10 min.

7. Carefully transfer the clear lysate to a clean 1.5 mL tube and add **1 volume** of **Buffer RET** (For example, 500 μL of Buffer RET to 500 μL of clear lysate), and 250 μL of 100% ethanol. Mix well by sharp hand shaking for 3 times.

8. Transfer **700 μL** of the lysate/ethanol mixture to the pretreated **Mini Column (White ring)** with a **2 mL Collection Tube** and centrifuge at 12,000 rpm for 30 s. Discard the flow-through liquid and transfer the remaining lysate/ethanol mixture to the column. Centrifuge at 12,000 rpm for 30 s and discard the flow-through, put the column back to the collection tube.

9. Add **500 μL Buffer KB** and centrifuge at 12,000 rpm for 30 s and put the 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, JM101 and their

derived strains.

10. Add **500 µL DNA Wash Buffer** *(Add ethanol to DNA Wash Buffer before use)* and centrifuge at 12,000 rpm for 30 s. Discard the flow-through and put the **Mini Column** back to the collection tube.

    Repeat step **10**.

11. Centrifuge the column at 12,000 rpm for 1 min.

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

12. Carefully transfer the spin column into a sterile 1.5 mL tube and add **50-100 µL** of **Endofree Elution Buffer**. Incubate for 1 min at room temperature and centrifuge at 12,000 rpm for 1 min .

    Optional : Reload the eluate into the column and centrifuge again to improve the recovery.

    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.

    Note: For protocol IV, this kit only provides a small amount of Buffer RET, if you need more, it is recommended to purchase our EndoFree Plasmid ezFlow Miniprep Kit (BW-PD1220) separately.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low DNA yield	1. Poor cell lysis. 2. Bacterial culture overgrown or not fresh. 3. Low copy-number plasmid.	1. Make fresh Buffer B1 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 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 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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