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EZgene™ EndoFree Plasmid ezFlow Miniprep Kit

（BW-PD1220）

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Kit Contents

Catalog#	BW-PD1220-00	BW-PD1220-01	BW-PD1220-02
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	3 mL	15 mL	70 mL
Buffer B1	3 mL	15 mL	70 mL
Buffer N3	1 mL	5 mL	24 mL
Buffer RET	3 mL	15 mL	70 mL
Buffer KB	6 mL	30 mL	135 mL
DNA Wash Buffer*	3 mL	12 mL	54 mL
EndoFree Elution Buffer	2 mL	10 mL	30 mL
RNase A(20 mg/mL)	15 µL	75 µL	350 µL
User Manual	1	1	1

*Add 12 mL(BW-PD1220-00) or 48 mL(BW-PD1220-01) or 216 mL(BW-PD1220-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

Key to the plasmid purification kit is our proprietary DNA binding system that allows the high efficient binding of DNA to our ezBind matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily eluted with sterile water or EndoFree Elution Buffer. The purified DNA is guanidine/anion exchange resin residues free.

The EZgene™EndoFree system uses a specially formulated buffer that extracts the endotoxin from the bacterial lysate. The endotoxin level is 1 to 10 EU (Endotoxin) per µg.

This kit is designed for fast and efficient purification of plasmid DNA from 1 to 5 mL of *E. coli* culture. The mini column has a DNA binding capacity of 50 µg. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection. For lower endotoxin level, EndoClean Buffer (Supplied in

PD1212 or purchase separately from Biomiga) could be used to remove residual endotoxin.

Important Information

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 accordingly. Please contact our customer service for further information and refer to Table 1 for the commonly used plasmids.

Table 1 Commonly used plasmids and expected yield.

Plasmid	Origin	Copy Numbers	Expected Yield (μg per 1 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
pGEM®	Muted pMB1	300-400	6-7
pBluescript®	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. The strains used for propagating plasmid have significant influence on yield. Host strains such as TOP10, DH5α and C600 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 recommended transform plasmid to an *endA*- strain if the yield is not satisfactory. Please refer to Table 2 for the *endA* information.

Table 2 *endA* strains of *E. coli*.

End A- Strains of *E.coli*							
DH5α	DH1	DH21	JM106	JM109	SK2267	SRB	XLO
TOP10	DH10B	JM103	JM107	SK1590	MM294	Stbl2™	XL1-Blue
BJ5182	DH20	JM105	JM108	SK1592	Select96™	Stbl4™	XL10-Gold
End A+ 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	BMH71-18
All NM Strains				All Y Strains			

Optimal Cell Mass (OD_{600} × mL of Culture): This procedure is designed for isolating plasmid

grown in standard LB medium (Luria Bertani) for 12-16 hours to a density of OD_{600} 2.0 to 3.0. If rich medium such as TB or 2 × YT 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 has an optimal biomass of 10-15. For example, if the OD_{600} is 2.5, the optimal culture volume should be 1-5 mL. For over amount of cell numbers, either reduce the biomass or scale up the volume of Buffer A1, B1, N3 and RET.

Culture Volume: Use a flask or tube 4 times bigger in volume than the culture medium to secure optimal condition for bacteria growth. 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 purity.

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

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: 20 mg/mL. It is stable for one year at room temperature (15-25℃). Spin down RNase A vial briefly before adding to Buffer A1.

✪ Buffer A1 should be stored at 4℃ once RNase A is added.

✪ Add 12 mL(BW-PD1220-00) or 48 mL(BW-PD1220-01) or 216 mL(BW-PD1220-02) 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℃ to dissolve the precipitates before use. Keep the cap tightly closed for Buffer B1 after use.

✪ Buffer N3 may form precipitates upon storage, warm up at 37℃ to dissolve the precipitates before use.

✪ Ensure the availability of centrifuge capable of 12,000 ×g.

✪ *Carry out all centrifugations at room temperature.*

Materials not Supplied

✪ High speed centrifuge.

✪ 96-100% ethanol.

✪ 1.5 mL centrifugal tubes.

Safety Information

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

Buffer 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 ezFlow Miniprep Spin Protocol

1. Inoculate ***1-5 mL*** LB containing appropriate antibiotic with 100 µL fresh starter culture. Incubate at 37℃ for 14-16 hours with vigorous shaking.

   Note: Prolonged incubation (>16 hours) is not recommended since the *E. coli* starts to lyse and the plasmid yield may be reduced.

   Note: Do not grow the culture directly from the glycerol stock.

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

   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.

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 the bacterial culture by centrifugation for 1 minutes at 12000 ×g. 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 **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.

5. Add **250 µL Buffer B1**, mix gently by inverting the tube 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 **75 µL Buffer N3**, mix completely by inverting/shaking the vial for 5-10 times.

   Note: Incubating the lysate in ice for 1 minute will improve the yield.

   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. Transfer the lysate to a high speed centrifuge tube and centrifuge at 12,000 ×g for 10 minutes at room temperature. Transfer the cleared lysate to a 1.5 mL centrifugal tube (avoid the floating

precipitates).

Note: If the rotor is cold, incubate the lysate at room temperature for 10 minutes and then perform centrifugation as described.

8. Add **250 µL Buffer RET** and **250 µL** 100% ethanol. Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be centrifuged through the DNA column immediately.

9. Immediately apply **700 µL** of the lysate/ethanol mixture to the **pretreated Mini Column** with the **2 mL Collection Tube**. Centrifuge at 12,000 ×g for 1 minute at room temperature. Discard the flow-through liquid and put the column back to the **2 mL Collection Tube**. Add the remaining lysate/ethanol mixture to the DNA column and centrifuge at 12,000 ×g for 1 minute. Discard the flow-through liquid and put the column back to the collection tube.

10. Add **500 µL Buffer KB** into the **Mini Column**, centrifuge at 12,000 ×g for 1 minute, discard the flow-through. 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.

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

Repeat step **11**.

12. Reinsert the column, with the lid open, into the **2 mL Collection Tube** and centrifuge for 2 minutes at 12,000 ×g.

Note: Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.

13. Carefully transfer the **Mini Column** into a sterile 1.5 mL centrifugal tube and add **50-100 µL (>50 µL)** sterile ddH_2O or **EndoFree Elution Buffer (preheating at 65°C)** into the center of the column and let it stand for 1 minute. Elute the DNA by centrifugation at 12,000 ×g for 1 minutes.

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.

Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, library

screening, *in vitro* translation, sequencing, transfection of robust cells such as HEK293 cells.

Note: It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

14. The DNA concentration can be calculated as follows,

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

Protocol (For spin/vacuum)

1. Set up the vacuum manifold according to manufacturer's instruction and connect the **Mini Column** to the manifold.

2. Carry out step **1-8** on page **6-7** in previous protocol.

3. Carefully transfer the lysate/ethanol mixture to the **Mini Column** and turn on the vacuum to allow the lysate pass through the column. Repeat until the remaining solution pass through the column.

4. **Optional:** Add **500 μL Buffer KB** into the **Mini Column** and allow the lysate pass through the column by vacuum.

   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.

5. Add **500 μL DNA Wash Buffer** to the **Mini Column** and allow the vacuum to draw the liquid through the manifold. Turn off the vacuum.

   Optional: Repeat step **5**.

6. Turn on the vacuum, dry the empty column for 5 minutes.

7. Carefully transfer the **Mini Column** into a clean 1.5 mL tube and add **50-100 μL (>50 μL)**sterile ddH_2O or **EndoFree Elution Buffer** into the center of the column and let it stand for 1 minute. Elute the DNA by centrifugation at 12,000 ×g for 1 minute.

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

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 **2×volumes** of the **Buffer A1, Buffer B1, Buffer N3 and Buffer RET**. 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 throughly by vortexing and pipetting prior to 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 and the volume of Buffer A1, B1, N3 and RET as instructed on page 9.
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	Ethanol traces were not completely removed from column.	Make sure that no ethanol residue remains 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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