Ver: 2409

EZgene™ 96-Well Plasmid Miniprep Kit

（BW-PD1812）

Contents

Kit Contents

Catalog#	BW-PD1812-00	BW-PD1812-01	BW-PD1812-02
Preps	96	96 x 4	96 x 20
96-Deep Well Plates (1.6 mL)	1	4	20
96-Well DNA Plates	1	4	20
96-Well Lysate Clearance Plates	1	4	20
96-Well Elution Plates	1	4	20
Breathable Films	1	4	20
Sealing Films	4	16	80
Buffer GBL	60 mL	240 mL	2 x 500 mL
Buffer A1	30 mL	110 mL	2 x 300 mL
Buffer B1	30 mL	110 mL	2 x 300 mL
Buffer N1	40 mL	160 mL	2 x 400 mL
DNA Wash Buffer*	40 mL	2 x 80 mL	8 x 100 mL
Buffer KB	55 mL	240 mL	3 x 400 mL
Elution Buffer	25 mL	100 mL	450 mL
RNase A(20mg/mL)	150 µL	550 µL	2 x 1.5 mL
Use Manual	1	1	1

*Add 160 mL (BW-PD1812-00) or 320 mL (BW-PD1812-01) or 400 mL (BW-PD1812-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

The EZgene™ 96-well plasmid miniprep kit provides an easy and fast method for isolating high quality plasmid DNA in a high through put format. The key to this system is Biomiga's ezBind matrix that avidly, but reversibly, binds DNA under optimized buffer condition while proteins and other unwanted contaminants are removed by wash buffer. High quality plasmid DNAs are eluted with TE buffer or deionized water. By using the 96-well kit, up to 96 samples can be simultaneously processed in less than 90 minutes. The 96-well lysate clearance plate obviates the time-consuming centrifugation step and increases the DNA recovery up to 20%. The purified DNA is ready for downstream applications such as cloning/subcloning, RFLP, sequencing, and transfection of robust cells such as HEK293 cells.

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 and DH5α 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. For example, if the OD_{600} is 3.0, 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 and N1.

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 160 mL (BW-PD1812-00) or 320 mL (BW-PD1812-01) or 400 mL (BW-PD1812-02) 96-100% ethanol to 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.

✪ *Carry out all centrifugations at room temperature.*

Materials not Supplied

✪ Robotic station if using automated isolation.

✪ Centrifuge with swing-bucket rotor (4,000 ×g).

✪ 96-Deep Well Plates (2.2 mL).

✪ Vacuum pump capable of achieving 300-400 mbar.

✪ Standard vacuum manifold.

✪ Oven or incubator preset to 70℃.

✪ 100% ethanol.

Safety Information

Buffer N1 contains chaotropic salts, 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.

Vacuum Manifold Protocol

1. Inoculate ***1-1.5 mL*** LB/antibiotics medium in a **96-Deep Well Plate (2.2 mL)** with *E.coli* carrying desired plasmid and grow at 37℃ for 14-16 hours.

   Note: The 96-Deep Well Plate (2.2 mL) needs to be purchased separately.

2. Column equilibration: Place the **96-Well DNA Plate** into the plate holder inside the manifold, and add **500 µL Buffer GBL** to column. Discard the flow-through, and set the plate back into the deep plate. (Please use freshly treated spin column).

3. Seal the plate with **Breathable Film** and pellet the bacterial by centrifugation at 1,500-2,000 ×g for 5 minutes in a swing-bucket rotor at room temperature. Remove the **Breathable Film** and discard supernatant. Tapping the inverted plate firmly in paper towel to remove excess medium.

   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** to each well, mix gently by inverting the tube 10 times (*do not vortex*), and incubate at room temperature for 2-3 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 **350 µL Buffer N1** to each well. Wipe off any buffer residues on the top of the plate and seal the plate with a **Sealing Film**. Mix by inverting the plate for 5 times and vortex for 5 seconds. The flocculent white precipitate should form.

7. Assemble the vacuum manifold:

a. Place the **pre-96-Well DNA Plate** into the plate holder inside the manifold;

b. Place the **96-Well Lysate Clearance Plate** on top of the manifold.

Note: Make sure to adjust the positions of samples between the 96-Well Lysate Clearance Plate and the 96-Well DNA Plate.

8. Immediately transfer the lysate into the **96-Well Lysate Clearance Plate**. Allow the lysate to

stand for 5-10 minutes. The white precipitate should float to the top. Apply vacuum until all the lysate passes through the **96-Well Lysate Clearance Plate**.

9. Turn off the vacuum and discard the **96-Well Lysate Clearance Plate**. Carefully transfer the **96-Well DNA Plate** that contains the cleared lysate to the top of the vacuum manifold and turn on the vacuum till all the lysate passes through the **96-Well DNA Plate**. (The **96-Deep Well Plate (1.6 mL)** now should be positioned under the 96-Well DNA Plate; some manifold may require internal height adjustment by other plate).

10. **Optional:** Add **500 µL Buffer KB** to each well and apply vacuum till all the liquid passes through the **96-Well DNA Plate**. Turn off the 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.

11. Add **750 µL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) to each well and turn on vacuum till all buffer passes through the plate. Turn off vacuum.

    Optional: Repeat step **11**.

12. Discard the waste in the manifold and dry the **96-Well DNA Plate** with maximum vacuum power for 20 minutes.

13. Remove the **96-Well DNA Plate** from the manifold and tap the plate on a stack of absorbent paper towels. Remove any residual moisture from the tip ends of the **96-Well DNA Plate** with clean paper towel.

    Optional: Place the **96-Well DNA Plate** into a vacuum oven preset at 70°C for 10 minutes.

14. Place the **96-Well DNA Plate** back to the vacuum manifold and apply maximum vacuum for another 5 minutes.

15. Place the **96-Well Elution Plate** inside the manifold with the manifold adaptor and set the **96-Well DNA Plate** on top of the manifold (Make height adjustment as necessary by adding another plate).

16. Add **100-150 µL Elution Buffer** or sterile water to each well, let plate stand for 2 minutes.

Apply maximun vacuum for 5-10 minutes to elute the DNA from the plate. Turn off vacuum and ventilate the manifold slowly.

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

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

Note: The DNA recovery rate and concentration depend on the elution volume. For maximum yields, elute with another 50 µL Elution Buffer although the DNA concentration will be lower.

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.

17. The DNA concentration can be calculated as follows,

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

Centrifuge Protocol

1. Inoculate ***1-1.5 mL*** LB/antibiotics medium in a **96-Deep Well Plate (2.2 mL)** with *E.coli* carrying desired plasmid and grow at 37℃ for 14-16 hours.

   Note: The 96-Deep Well Plate (2.2 mL) needs to be purchased separately.

2. Column equilibration: Place a 96-Well DNA Plate in a 96 well deep plate, and add **500 µL Buffer GBL** to column. Centrifuge for3 min at 3000 xg in a table-top centrifuge. Discard the flow-through, and set the plate back into the deep plate. (Please use freshly treated spin column).

3. Seal the plate with **Breathable Film** and pellet the bacterial by centrifugation at 1,500-2,000 ×g for 5 minutes in a swing-bucket rotor at room temperature. Remove the **Breathable Film** and discard supernatant. Tapping the inverted plate firmly in paper towel to remove excess medium.

   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** to each well, mix gently by inverting the tube 10 times (*do not vortex*), and incubate at room temperature for 2-3 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 **350 µL Buffer N1** to each well. Wipe off any buffer residues on the top of the plate and seal the plate with a **Sealing Film**. Mix by inverting the plate for 5 times and vortex for 5 seconds. The flocculent white precipitate should form.

7. Place the **96-Well Lysate Clearance Plate** on top of a **96-Deep Well Plate (1.6 mL)**. Transfer the lysate into the **96-Well Lysate Clearance Plate** and allow the lysate to sit for 10 minutes. White precipitate should float to the top at this point.

8. Place the clearance/deep well plates in a swing-bucket rotor and centrifuge at 2,000 ×g for 5

minutes. Discard the **96-Well Lysate Clearance Plate**.

9. Place a **pre-96-Well DNA Plate** on top of a **96-Deep Well Plate (1.6 mL)** and transfer the cleared lysate into the **96-Well DNA Plate**.

10. Centrifuge at 3,000 ×g for 5 minutes. Discard the flow-through liquid and reuse the **96-Deep Well Plate (1.6 mL)** for next step.

11. **Optional:** Add **500 µL Buffer KB** to each well and centrifuge at 3,000 ×g for 5 minutes. Discard the flow-through liquid and reuse the **96-Deep Well Plate (1.6 mL)** for next step.

    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.

12. Add **750 µL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) to each well and centrifuge at 3,000 ×g for 10 minutes. Discard the flow-through liquid and reuse the **96-Deep Well Plate (1.6 mL)** for next step.

    Repeat step **12**.

13. Place the **96-Well DNA Plate** on top of a **96-Deep Well Plate (1.6 mL)** and centrifuge at 3,000 ×g for 10 minutes.

    Optional: Place the **96-Well DNA Plate** into a vacuum oven preset at 70°C for 10 minutes.

14. Place the **96-Well DNA Plate** on the top of a **96-Well Elution Plate**. Add **100-150 µL Elution Buffer** or sterile water to each well of the **96-Well DNA Plate**, let plate stand for 2 minutes. Centrifuge the plate at 3,000 ×g for 10 minutes to elute the DNA.

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

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

    Note: The DNA recovery rate and concentration depend on the elution volume. For maximum yields, elute with another 50 µL Elution Buffer although the DNA concentration will be lower.

    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.

15. The DNA concentration can be calculated as follows,

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

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** and **Buffer N1**. Additional buffers can be purchased from Biomiga.

✪ Use **same volume** of **DNA Wash Buffer** and **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,N1 as instructed on page 11.
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 flow out of agarose gel during loading	Trace ethanol contamination.	Wash the plate as instructed.
OD doesn't match the DNA yield on agarose gel	Trace ethanol contamination.	Wash the plate as instructed.
96-Well Lysate Clearance Plate clogged	Lysate was not mixed well after adding Buffer N1.	Mix the lysate well by inverting the plate for 5 times and then vortex for 10 seconds.

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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