Ver: 2404

EZgene™ EndoFree Plasmid Maxiprep Kit

（BW-PD1514）

Contents

Kit Contents

Catalog#	BW-PD1514-00	BW-PD1514-01	BW-PD1514-02
Preps	2	10	25
Maxi Columns	2	10	25
Collection Tubes	2	10	25
Buffer GBL	6 mL	30 mL	75 mL
Buffer A1	22 mL	110 mL	270 mL
Buffer B1	22 mL	110 mL	270 mL
Buffer N3	27 mL	130 mL	2 x 170 mL
DNA Wash Buffer*	5 mL	24 mL	54 mL
EndoClean Buffer	5 mL	25 mL	60 mL
EndoFree Elution Buffer	5 mL	25 mL	60 mL
RNase A (20 mg/mL)	110 µL	550 µL	1.35 mL
User Manual	1	1	1

*Add 20 mL (BW-PD1514-00) or 96 mL (BW-PD1514-01) or 216 mL (BW-PD1514-02) 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 Elution Buffer. Unlike other kits in the markets, our patented plasmid purification kit has no chaotropic salts in the 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. Two rounds of extraction will reduce the endotoxin level to 0.1 EU (Endotoxin) per µg of plasmid DNA.

This kit is designed for fast and efficient purification of plasmid DNA from 150 to 200 mL of *E. coli* culture. The Maxi Column has a DNA binding capacity of 1 mg. The purified endofree

DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Two endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA and Protocol B removes endotoxin after the purification of plasmid DNA.

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 200 mL)
pSC101	*pSC101*	5	12
pACYC	*p15A*	10-12	25-40
pSuperCos	*pMB1*	10-20	30-50
pBR322	*pMB1*	15-20	35-50
pGEM^{R}	*Muted pMB1*	300-400	350-450
pBluescript^{R}	*ColE1*	300-500	450-600
pUC	*Muted pMB1*	500-700	700-1,000

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*^{TM}	*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 Maxi Column has an optimal biomass of 400-500. For example, if the OD_{600} is 2.5, the optimal culture volume should be 150 to 200 mL. For over amount of cell numbers, either reduce the biomass or scale up the volume of Buffer A1, B1 and N3.

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

Alternative endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA and Protocol B removes endotoxin after the purification of plasmid DNA.

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 20 mL (BW-PD1514-00) or 96 mL (BW-PD1514-01) or 216 mL (BW-PD1514-02) 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.

✪ Ensure the availability of centrifuge capable of 8,000 ×g.

✪ *Carry out all centrifugations at room temperature.*

Materials not Supplied

✪ High speed centrifuge.

✪ 96-100% ethanol.

✪ 50 mL centrifugal tubes.

✪ Isopropanol.

Safety Information

Buffer N3 contains acidic acid, wear gloves and protective eyewear when handling. Do not add bleach or acidic solutions directly to the preparation waste.

EZgene™ EndoFree Plasmid Maxiprep Spin Protocol

A. Removal of endotoxin during plasmid purification

This protocol is designed for removing the endotoxin during the plasmid purification.

1. Inoculate ***150-200 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: Do not use more than 200 mL culture or cell mass greater than 550. The buffer volume needs to be scaled up if processing over 200 mL of culture.

   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 Maxi Column in a clean collection tube, and add **2.5 mL** Buffer GBL to Maxi Column. Centrifuge for 2 min at 8,000×g in a table-top centrifuge. Discard the flow-through, and set the Maxi Column back into the collection tube. (Please use freshly treated spin column).

3. Harvest the bacterial culture by centrifugation for 10 minutes at 5,000 ×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 **10 mL 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 **10 mL 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 **3 mL 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: If the amount of RNA in the bacterial solution is large, it can be allowed to stand for 10 minutes to make the RNase fully function.

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 8,000 ×g for 10 minutes at room temperature. Transfer the cleared lysate to a 50 mL centrifugal tube (avoid the floating precipitates).

   Note: If high-speed centrifuge is not available, Syringe Filter (can be purchased from Biomiga separately) can be used to clear the lysate.

8. Carefully transfer the clear lysate to a new high-speed centrifuge tube, avoid the precipitations. Add **0.1 volume EndoClean Buffer**, vortex for 5 seconds and incubate on ice for 10 minutes, mix the sample several times without leaving ice.

   Note: Use a serological pipet or a tip cut with a clean razor in the end to transfer the EndoClean Buffer.

   Note: Mix the sample several times during incubation without leaving ice.

   Note: At room temperature (>23°C), the sample becomes turbid after adding EndoClean Buffer. The solution becomes clear after incubating on ice.

9. Incubate the solution at 65°C for 5 minutes. The solution becomes turbid. Centrifuge the sample at 10,000 ×g for 10 minutes (*Alternatively, the sample can be centrifuged at 2,500 ×g for 20 minutes*) at room temperature (*the temperature must be higher than 23°C*). The solution should separate into 2 phases, the upper clear phase contains DNA and the lower organic phase contains endotoxin. The two phases will not separate if the temperature is below 23°C.

   Note: The red color in the upper phase will not affect the result.

   Note: Up to 99% of the endotoxin can be removed by extracting with the EndoClean Buffer once. The endo level is in the range of 0.1 to 10 EU (Endotoxin) per µg of DNA. Another extraction is necessary if less than 0.1 EU/µg of DNA is desired by repeating step 7-8.

10. Carefully transfer the clear supernatant into a 50 mL centrifugal tube (avoid the floating precipitates). Add **9 mL Buffer N3** and **10 mL** 100% ethanol. Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be centrifuged through the DNA column immediately.

11. Immediately apply **18 mL** of the lysate/ethanol mixture to a **pretreated Maxi Column** with the collection tube. Centrifuge at 8,000 ×g for 1 minute at room temperature. Discard the

flow-through liquid and put the column back to the collection tube. Add the remaining lysate/ethanol mixture to the DNA column and centrifuge at 8,000 ×g for 1 minute. Discard the flow-through liquid and put the column back to the collection tube.

Note: The Maxi column has a maximum capacity of 20 mL. If apply 20 mL of the lysate/ethanol mixture to the Maxi Column, you should incubate 2-5 minutes at room temperature (avoid splashing of the mixture during centrifugation).

12. Add **10 mL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **Maxi Column**, centrifuge at 8,000 ×g for 1 minute, discard the flow-through.

13. Add **10 mL** 100% ethanol into the column, centrifuge at 8,000 ×g for 1 minute, discard the flow-through.

14. Reinsert the column, with the lid open, into the 50 mL centrifugal tube and centrifuge for 10 minutes at 10,000 ×g.

    Note: It is critical to remove residual ethanol completely. Residual ethanol can be removed more efficiently with 50-60℃ for 10 minutes after centrifuge.

15. Carefully transfer the **Maxi Column** into a clean 50 mL **Collection Tube** and add **1.5-2 mL** sterile ddH_2O or **EndoFree Elution Buffer (preheating at 55℃)** into the center of the column and let it stand for 1 minute. Elute the DNA by centrifugation at 10,000 ×g for 5 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 and microinjection.

16. The DNA concentration can be calculated as follows,

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

B. Removal of endotoxin after plasmid purification

This protocol is designed for removing the endotoxin after the plasmid purification.

1. Follow the protocol on Page 6-7 from **Step 1 to 6**.

2. Transfer the lysate to a sterile 50 mL centrifugal tube and add **9 mL Buffer N3** and **10 mL** 100% ethanol. Mix well and go to **Step 11-15** on Page 7-8.

3. After the plasmid is purified, add **0.1 volume EndoClean Buffer** to the plasmid sample in a 2 mL microfuge tube (For example, add **0.1 mL EndoClean Buffer** to **1 mL plasmid sample**).

   Note: The solution becomes turbid after adding EndoClean Buffer. If the temperature is below 23℃, the solution remains clear.

   Note: Use a clean razor to cut the tip end to transfer the viscous EndoClean Buffer.

4. Vortex the sample for 10 seconds and incubate the tube on ice for 10 minutes. Mix the sample several times without leaving ice. The solution becomes clear after incubating on ice.

5. Incubate the solution at 65℃ for 5 minutes. The solution becomes turbid again. Centrifuge at 12,000 ×g at room temperature for 10 minutes (the temperature must be higher than 23℃). The two phases will not separate if the temperature is below 23℃.

6. Carefully transfer the upper clear layer solution to another 2 mL microfuge tube. Precipitate plasmid DNA with **0.1 volume** 3 M KAc (pH 5.2) and **0.7 volume** Isopropanol. Centrifuge at 12,000 ×g for 10 minutes. Carefully decant.

7. Add **1 mL** 70% ethanol and centrifuge at 12,000 ×g for 5 minutes. Carefully decant and air-dry the DNA for 30 minutes in a hood.

8. Resuspend the DNA with **EndoFree Elution Buffer**.

   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, and microinjection.

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 **400 mL** for the maxiprep.

✪ Use **2×volumes** of the **Buffer A1, Buffer B1** and **Buffer N3** and **100% ethanol**. 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 according to instruction on page 10.
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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