Ver: 2404

EZgene™ Plasmid ezFilter Midiprep Kit II
(BW-PD1414）

Contents

Kit Contents

Catalog#	BW-PD1414-00	BW-PD141401	BW-PD1414-02
Preps	2	10	25
EzBind™ Columns	2	10	25
Filter Syringe (20 mL)	2	10	25
Buffer GBL	7.5 mL	30 mL	70 mL
Buffer A1	11 mL	55 mL	135 mL
Buffer B1	11 mL	55 mL	135 mL
Buffer C1	14 mL	70 mL	170 mL
DNA Wash Buffer*	5 mL	24 mL	54 mL
Elution Buffer	4 mL	20 mL	60 mL
RNase A (20 mg/mL)	55 µL	275 µL	675 µL
User Manual	1	1	1

*Add 20 mL (PD1414-00) or 96 mL (PD1414-01) or 216 mL (PD1414-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

Key to the kit is our proprietary DNA binding systems that allow the highly efficient binding of DNA to our ezBindTM 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, no chaotropic salts are contained in the buffer of our patented plasmid purification kit. The purified DNA is guanidine/anion exchange resin residues free.

This kit is designed for fast and efficient purification of plasmid DNA from 50 to 100 mL of E. coli culture. The midi column has a plasmid DNA binding capacity of 400 µg.

The purified DNA is ready for high performance of downstream applications such as transfection of robust cells such as HEK293, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations.

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 100 mL)
pSC101	*pSC101*	5	5
pACYC	*p15A*	10-12	10-12
pSuperCos	*pMB1*	10-20	10-20
pBR322	*pMB1*	15-20	15-20
pGEM^{R}	*Muted pMB1*	300-400	300-400
pBluescript^{R}	*ColE1*	300-500	300-500
pUC	*Muted pMB1*	500-700	500-700

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 Midi Column II has an optimal biomass of 150-250. For example, if the OD_{600} is 2.5, the optimal culture volume should be 50 to 100 mL. For over amount of cell numbers, either reduce the biomass or scale up the volume of Buffer A1, B1 and C1.

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: It is stable for more than half a year when stored at room temperature. Spin down the RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use. Store at 4°C.
- Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.
- Buffer C1 may form precipitates below 10°C, warm up at 37°C to dissolve the precipitates before use .
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure the availability of centrifuge (13,000 rpm). Especially, after mixing the lysate with ethanol, the sample needs to be processed immediately either by centrifugation.
- *Carry out all centrifugations at room temperature.*

Materials not Supplied

- 100% ethanol
- High speed centrifuge
- 30 mL high speed centrifuge tubes
- 50 mL conical tubes
- Isopropanol if precipitate the plasmid DNA.

Safety Information

- Buffer C1 contains acidic acid, wear gloves and protective eyewear when handling.
- Buffer C1 contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

EZgene™ Plasmid ezFilter Midiprep II Spin Protocol

1. Inoculate **50-100 mL** LB containing appropriate antibiotic with 50 μL fresh starter culture. Incubate at 37 ℃for 14-16 hours with vigorous shaking.

   Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 mL LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 hours with vigorous shaking (~250 rpm).

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

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

   Note: Do not use more than 50 mL culture or cell mass greater than 150.

2. Column equilibration: Place a **EzBind™ Column** in a clean collection tube, and add **2.5mL Buffer GBL** to column. Centrifuge for 1 min at 8000 rpm in a table-top centrifuge. Discard the flow-through, and set the 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 **5 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 **5 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°C to dissolve the precipitations before use.

6. Add **6 mL Buffer C1**, 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. **Optional 1:** Transfer the lysate to a high speed centrifuge tube and centrifuge at 8000 rpm for 10 minutes at room temperature. Carefully transfer the clear supernatant into a 50 mL 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.

Optional 2: Pour the lysate directly into the barrel of the filter syringe. Insert the syringe to a clean 50 mL tube (not supplied) set in a rack. Allow the cell lysate to sit for 10 minutes. The white precipitates should float to the top. Hold the filter syringe barrel over the 50 mL tube and gently insert the plunger to expel the cleared lysate to the tube, stop when feel major resistance, some of the lysate may remain in the flocculent precipitate, do not force the residual lysate through the filter.

8. Carefully transfer the clear supernatant into a 50 mL tube (avoid the floating precipitates). Add **6 mL 100% ethanol.** Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be transferred to the pretreatment EzBind™ Column immediately.

9. Immediately transfer the lysate/ethonal mix into a pretreatment EzBind™ Column with the collection tube. Centrifuge at 8000 rpm for 1 minutes at room temperature. Remove the column from the tube and discard the flow-through liquid. Reinsert the column to the collection tube. Repeat step 8 till all the lysate/ethonal mix has been passed through the column.

10. Add **5 mL DNA Washing Buffer** into the EzBind™ Column, centrifuge at 8000 rpm for 1 minutes. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step "9".

11. Add **3 mL 100% ethanol** into the EzBind™ Column, centrifuge at 8000 rpm for 1 min. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube.

12. Centrifuge the EzBind™ Column, **with the lid open**, at 8000 rpm for 10 minutes to remove the ethanol residues. Incubate at 65ºC for 10 min will help to remove the ethonal and increase the elution efficiency.

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

13. Carefully transfer the spin EzBind™ Column to a sterile clean tube and add **0.5-1 mL ddH$_2$O or Elution Buffer** to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 8000 rpm for 5 minutes.

14. For higher yield, reload the elute in the 50 mL tube to the columnand incubate for 1 min. Elute the DNA again by centrifugation at 8000 rpm for 5 min.

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

screening, *in vitro* translation, sequencing, transfection of 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.

Note: If ddH_2O is used for elution, make sure that the pH is between 7.0 and 8.5. pH lower than 7 leads to lower elution efficiency.

Note: Two elutions give rise to maximum DNA yield. For maximum yield and higher concentration, pool the elutions together, add 0.1 volume 3M KAc or NaAc (pH 5.2) and 0.7 volume isopropanol. Mix well and aliquot the sample to 2.0 ml microtubes. Centrifuge at top speed for 10 min. Remove the supernatant. Wash the DNA with 800µL 70% ethanol, centrifuge for 5 min, carefully decant. Air-dry the pellet for 5-10 min. Resuspend the DNA in Elution Buffer or sterile ddH_2O.

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 **100 mL** for the midiprep.

✪ Use **2×volumes** of the **Buffer A1, Buffer B1** and **Buffer C1**. Additional buffers can be purchased from Biomiga.

✪ Use **same volume** of **DNA Wash Buffer** and **Elution Buffer**.

质粒中提过滤法 II 型简明步骤（PD1414）

（详细内容请参考说明书英文部分）

I. 实验前准备

RNase A:室温下可稳定保存半年，长期贮藏请置于4oC保存。使用前将提供的所有RNase A瞬时离心后加入Buffer A1，使用后将Buffer A1/RNase A置于4°C保存。

Buffer B1: 在低于室温时会沉淀，请于50°C左右水浴加热至沉淀完全溶解，溶液澄清，使用后保证Buffer B1瓶盖旋紧。

Buffer C1：低于10°C会沉淀，请于37°C左右水浴加热至沉淀完全溶解，溶液澄清。

准备100%的乙醇。在室温下（22-25°C）进行所有离心操作。

II. 注意事项

质粒拷贝数： 纯化中低拷贝的质粒时， 使用 2 倍的菌液体积， 2 倍的Buffer A1,B1,C1,100%乙醇，相同体积的DNA Washing Buffer 和Endofree Elution Buffer..

转化菌: 若为-70°C甘油冻存的菌，请先涂布平板培养后，再重新挑选新的单个菌落进行培养。切勿直接取冻存在4°C的菌进行培养。

柱结合能力：450 μg。

III. 操作步骤

1. 取50 μL新鲜的菌液接种到**50-100 mL** (勿超过 50 mL)的LB培养基（含适量抗生素），37°C震荡培养14-16小时。室温下5,000 x g离心10分钟，收集菌体，并尽可能的吸去上清。

   注：残留的液体培养基容易导致菌液裂解不充分，离心后沉淀较松，不能有效吸取上清。

   注：本说明书中的操作程序适用于标准 LB （Luria Bertani）培养基培养 12-16 小时后，OD600（细菌密度）在 2.0-3.0 之间的菌液。若采用的是富集培养基，例如 TB 或 2×YT，请注意保证 OD600 不超过 3.0。

2. 柱平衡：向吸附柱EzBind™ Column 中加入**2.5mL**平衡液 **Buffer GBL**，8000 rpm 离心1分钟，弃去收集管中的滤液，将吸附柱重新放回收集管中备用。（处理完请于当天使用）

3. 加入**5 mL Buffer A1**（确保已加入RNase A），用移液器或涡流震荡确保细菌沉淀重新悬浮。

   注：不完全悬浮易导致菌体裂解不完全，从而使产量降低。

4. 加入 **5 mL Buffer B1**, 轻轻地反转5-10次以混合均匀，然后静置2-5分钟至溶液粘稠而澄清。

注：切勿剧烈振荡。静置时间不应超过5分钟，时间过长会导致基因组DNA污染或质粒受到破坏。若溶液未清亮澄清，则表明菌体裂解不充分，应加大Buffer B1的用量或减少菌体量。

5. 加入**6 mL Buffer C1**，立即反转5次，用手用力摇晃3-5次充分混匀，此时出现白色絮状沉淀。
6. **方法一：**将离心管转至高速离心机，在室温下8000 rpm 离心10分钟（若上清中有白色沉淀，可再次离心）小心吸取离心后的上清液至50 mL管中（避免吸起沉淀）。

   注：低温下RNase不工作，易有RNA污染。如果离心机转子较冷，将离心管在室温下温育10分钟后再离心。

   方法二：将裂解液转移至过滤器中，放在一个50 mL的试管上静置10分钟。管中的白色絮状沉淀浮上来，对准50 mL的管向下压，使裂解液尽可能多的通过，有些裂解液可能会残留在沉淀中。

   注：静置10 分钟时RNase A将工作，排除RNA污染。
7. 加入**6.0 mL 100% 乙醇**，立即混匀，需马上离心过吸附柱。
8. 立即转移**6.0 mL**裂解液至带收集管的预处理吸附柱中，室温下8000 rpm 离心1分钟，倒掉收集管中的废液，将吸附柱重新放回到收集管中。重复此步直至所有的溶液通过吸附柱。
9. 向吸附柱加入**5.0 mL DNA Washing Buffer**，室温下>2,500 x g 离心1 分钟，倒掉收集管中的废液，将E吸附柱重新放回到收集管中。重复步骤“8”。
10. 向吸附柱中加入**3 mL 100%乙醇**，室温下8000 rpm 离心1分钟，倒掉收集管中的废液，吸附柱重新放回到收集管中。
11. 将吸附柱放回高速离心机中，室温下8000 rpm开盖离心10分钟。离心后，将离心柱置于65度烘箱中放置10min，有利于去除乙醇。

    注：此步骤中开盖离心将会更有效的去除残留的乙醇，乙醇是否去除干净将会影响最后的洗脱效率。
12. 将吸附柱转至一个新的15 mL离心管中，向吸附柱膜的正中加入**0.5-1 mL灭菌 ddH$_2$O（pH在7.0-8.5之间）**或**Elution Buffer**， 室温放置1分钟，>2,500 x g离心5分钟，以洗脱质粒DNA。若想提高得率，将50mL离心管中的洗脱液再加到吸附柱的中间，8000 rpm 离心5分钟以洗脱质粒DNA。

    注：提取到的质粒DNA可直接用于基因克隆、测序、酶切、文库筛选、体外转录翻译、转染HEK293细胞。若用于转染内毒素敏感性细胞株，原代细胞及用于微量建议去除内毒素（PD1415）。

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low Yield	Poor Cell lysis.	• Resuspend pellet thoroughly by votexing and pipetting prior adding Buffer B1. • Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2N 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 °C. if the culture is not purified the same day. Do not store culture at 4 °C over nig ht.
Low Yield	Low copy-number plasmid.	Increase culture volume and increase the volume of Buffer A1, B1, C1, and 100% ethanol according to instructions 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, DNA doesn't freeze or smell of ethanol	Ethanol traces 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 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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