Table of Contents

Contents……... 1

Introduction.....……. 2

Important Notes.....…….… 2

Storage and Stability.....…… 4

Before Starting.....…… 4

Kit Contents.....…… 5

Safety Information…….. 5

EZgene™ Plasmid Miniprep II Spin Protocol.....…….. 6

EZgene™ Plasmid Miniprep II Spin/Vacuum Protocol.....……... 8

Purification of Low-Copy-Number Plasmid/Cosmid……. 9

质粒小提试剂盒II简明步骤……. 10

Trouble Shooting Guide.....…… 12

Introduction

Key to the kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our ezBind™ matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or Elution Buffer.

This kit is designed for fast and efficient purification of plasmid DNA from 1 to 15 mL of E. coli culture. With the binding capacity of 80 μg, the yield obtained by Miniprep Kit II (PD1213) is higher than Miniprep Kit I (PD1211). The yield from 1 mL culture is typically around 8 to 12 μg.

The purified DNA is ready for downstream applications such as cloning/subcloning, RFLP, sequencing, and transfection of HEK293 cells.

Important Notes

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 3 to 5 times. Please reference Table 1 for the commonly used plasmids,

Table 1 commonly used plasmid 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. Host strains such as Top 10 and DH5a 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 recommend transform plasmid to an *endA*- strain if the yield is not satisfactory.

Table2 *endA*- strains of *E. Coli.*

EndA*- 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

EndA*+ 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	BMH 71-18
All NM strains				All Y strains			

Optimal Cell Mass (OD_{600} x 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 mediums such as TB or 2xYT 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 30-45. For example, if the OD600 is 3.0, the optimal culture volume should be 10-15 mL. For over amount of cell numbers, either reduce the biomass or scale up the volumes of Buffer A1, B1 and N1.

Culture Volume: Use a flask or tube 4 times bigger in volumn thanthe 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 less 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 (22-25℃). The Guaranteed shelf life is 12 months from the date of purchase.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important

- RNase A: It is stable for more than half a year when stored at room temperature. Spin down RNase A vial briefly. Add the RNase A solution to buffer A1 and mix well before use. Store at 4℃.
- Add 8 mL (PD1213-00) or 60 mL (PD1213-01) or 96 mL (PD1213-02) or 60 mL (PD1213-03) 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 50℃ to dissolve the precipitates before use.**
- Keep the cap tightly closed for Buffer B1 after use.
- Ensure the availability of centrifuge capable of 13,000 rpm.
- ***Carry out all centrifugations at room temperature.***

Materials supplied by users

- 96-100% ethanol
- 1.5 mL, 2.0 mL microcentrifuge tubes.
- 15 mL conical tubes.
- High speed microcentrifuge or Vacuum manifold.

Kit Contents

Catalog#	PD1213-00	PD1213-01	PD1213-02	PD1213-03
Preps	4	50	250	100
ezBind Columns	4	50	250	100
Buffer A1	2.5 mL	25 mL	125 mL	50 mL
Buffer B1	2.5 mL	25 mL	125 mL	50 mL
Buffer N1	3 mL	30 mL	135 mL	60 mL
Buffer KB	3 mL	30 mL	135 mL	60 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL	2 x 15 mL
Elution Buffer	1 mL	15 mL	60 mL	30 mL
RNase A (20 mg/mL)	0.25 mg (12.5 µL)	2.5 mg (125 µL)	12.5 mg (625 µL)	5 mg (250 µL)
User Manual	1	1	1	1

*Add 8 mL (PD1213-00) or 60 mL (PD1213-01) or 96 mL (PD1213-02) or 60 mL (PD1213-03) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Safety Information

- Buffer N1 contains acidic acid, wear gloves and protective eyewear while handling.
- Buffer N1 and KB 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 Miniprep II Spin Protocol

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 **Elution Buffer**.

1. Inoculate **5-12 mL LB** containing appropriate antibiotic with a fresh colony. 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 yields may be reduced.
   Note: Do not grow the culture directly from the glycerol stock.
   Note: This protocol is optimized for E. coli strain cultured in LB medium. When using TB or 2xYT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD600). Buffers need to be scaled up proportionally if over amount of cultures are being processed.

2. Harvest bacterial culture by centrifugation for 1 min at 10,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely.

   Note: The culture can be centrifuged at 6,000 rpm in a 15 mL conical tube for 10 minutes if high speed centrifuge tubes are not available. Alternatively, the cultures can also be spin down in multiple 2.0 mL tubes.

3. Add **450 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 **450 µL Buffer B1**, mix gently by inverting 10 times (do not vortex) and incubate at room temperature for 5 minutes. If necessary, continue inverting the tube until the solution becomes slightly clear.

   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 precipitation before use.

5. Add **550 µL Buffer N1**, mix completely by inverting/shaking the vial for 5 times and sharp hand shaking for 3 times.

   Note: Incubating the lysate in ice for 1 min 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.

6. Centrifuge the lysate at 13,000 rpm for 10 minutes at room temperature.

   Note: If the lysate doesn't appear clean, reverse the tube angle, centrifuge for 5 more

minutes and then transfer the clear lysate to DNA column.

7. Carefully transfer up to **700 µL** clear lysate into a DNA column with a collection tube, avoid the precipitations, spin at 13,000 rpm for 1 minute, discard the flow-through and put the column back to the collection tube. Carefully transfer the remaining clear lysate to the column and centrifuge at 13,000 rpm for 1 minute at room temperature and discard the flow-through in the collection tube. Put the column back to the collection tube.

8. **Optional:** Add **500 µL Buffer KB** into the spin column, centrifuge at 13,000 rpm for 1 minute. Remove the spin column from the tube and discard the flow-through. Put the column back to the collection tube.

   Note: Buffer KB is recommended for *endA*+ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from *endA*- strains such as Top 10 and DH5a. Please reference Table 2 on page 3.

9. Add **650 µL DNA Wash Buffer** *(Add ethanol to DNA wash buffer before use)* into the spin column, centrifuge at 13,000 rpm for 1 minute at room temperature. Remove the spin column from the tube and discard the flow-through. Repeat step "9" to improve the recovery.

10. Reinsert the spin column, **with the lid open**, into the collection tube and centrifuge for 2 minutes at 13,000 rpm.

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

11. Carefully transfer the spin column into a clean 1.5 mL tube and add **100-150 µL ddH_20** or **Elution Buffer** into the center of the column and let it stand for 2 minute. Elute the DNA by centrifugation at 13,000 rpm for 1 minute. Reload the eluate into the column and centrifuge again to improve the recovery.

    Note: The pH of Elution Buffer or ddH_2O will affect the plasmid DNA elution. If ddH_2O is applied, please make sure the pH is no less than 7.0 (7.0-8.5 is preferred). NaOH could be used to adjust the pH of ddH_2O.
    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 (PD1214) if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

12. The DNA concentration can be calculated as,
    Concentration (µg/mL) = OD_{260} nm x 50 x dilution factor.

EZgene™ Plasmid Miniprep II Spin/Vacuum Protocol

1. Set up the vacuum manifold according to manufacture's instruction and connect the column to the manifold.

2. Carry out step 1-6 on Page 6 in previous protocol.

3. Carefully transfer the clear lysate to the DNA column and turn on the vacuum to allow the lysate pass through the column.

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

   Note: Buffer KB is recommended for *endA*+ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from *endA*- strains such as Top 10 and DH5a. Please reference Table 2 on page 3.

5. Add **650 µL** of **DNA Wash Buffer** to the column and allow the vacuum to draw the liquid through the manifold. Turn off the vacuum. Repeat step "5".

6. Transfer the column, **with the lid open,** to a 2 mL collection tube and centrifuge at 13,000 rpm for 2 minutes.

7. Carefully transfer the spin column into a clean 1.5 mL tube and add **100-150 µL ddH$_2$0** or **Elution Buffer** into the column and let it stand for 1 minute. Elute the DNA by centrifugation at 13,000 rpm for 1 minute. Reload the eluate into the column and centrifuge again to improve the recovery.

   Note: The DNA is ready for downstream applications such as cloning, RFLP, library screening, *in vitro* translation, sequencing and transfection of robust cells such as HEK293 cells.

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:

1. Culture volume: Use **2 x volumes** of the high copy number culture. Use 25 mL for the miniprep II.

2. Use **2 x volumes** of the **Buffer A1**, **Buffer B1** and **Buffer N1**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **Wash Buffer (DNA Wash Buffer)** and **Elution Buffer**.

Purification of plasmid > 12 kb

For isolating plasmid DNA > 12 kb, use the following guideline:

1. Culture volume: Use **2 x volumes** of the culture.

2. Use **2 x volumes** of the **Buffer A1**, **Buffer B1** and **Buffer N1**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **Wash Buffer (DNA Wash Buffer)** and **Elution Buffer**.

4. Pre-warm the **Elution Buffer** at 65-70 ℃ and let the column stand for 5 mins after adding **Elution Buffer.**

质粒小提试剂盒 II 简明步骤（PD1213）

（详细内容请参考说明书英文部分）

I. 实验前准备

RNase A: 室温下可稳定贮藏半年，长期贮藏请置于4°C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。

DNA Wash Buffer*: 使用前请将8 mL (PD1213-00)或60 mL (PD1213-01)或96 mL (PD1213-02) 或 60 mL (PD1213-03)96-100%乙醇加入DNA Wash Buffer。

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。

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, N1, 相同体积的 DNA Wash Buffer 和 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。

切勿直接取冻存的菌种进行培养。

III. 操作步骤

若用于提取1-4 mL的菌落，请将**Buffer A1, B1, N1**的量降低至**250 µL, 250 µL** 及 **350 µL**，**DNA wash Buffer**及**Elution Buffer**的量不变。

1. 接种新鲜的单个菌落到**5-12 mL**的LB培养基（含适量抗生素），37°C震荡培养14-16小时（勿超过16小时）。室温下10,000 rpm离心1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体裂解不充分，第 5 步离心后沉淀较松，不易吸取上清。

   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，OD_{600}（细菌密度）在 2.0-3.0 之间的菌液。若采用的是富集培养基，如 TB 或 2×YT，注意保证 OD_{600} 不超过 3.0。

2. 加入**450 µL Buffer A1**（确保已加入RNase A），用移液器或涡流震荡充分悬浮细菌细胞。

   注：菌体如果没有充分悬浮均匀，将导致菌体裂解不完全，从而使产量降低。

3. 加入 **450 µL Buffer B1**, 轻轻地反转多次至溶液充分混匀，室温静置2-

5分钟至溶液粘稠而澄清。

注：切勿剧烈振荡。静置时间不超过5分钟，时间过长会导致基因组DNA污染或质粒受到损伤。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。

4. 加入**550 μL Buffer N1**, 立即反转多次，至溶液充分混匀，此时出现白色絮状沉淀。

5. 将离心管转至高速离心机，在**室温下**13,000 rpm离心10分钟（若上清中有白色沉淀，可再次离心）。

6. 小心吸取**700 μL**离心后的上清液至带有收集管的DNA柱中（避免吸起沉淀），室温下13,000 rpm 离心1分钟，倒掉收集管中的废液，将离心柱重新放回到收集管中。重复步骤"7"至全部上清离心过DNA柱。

7. **可选:** 向DNA柱中加入 **500 μL Buffer KB**, 室温下13,000 rpm 离心1分钟，倒掉收集管中的废液，将离心柱重新放回到收集管中。

注：此步对富含内源核酸酶的宿主菌（*endA*＋）来说是必须的，如HB101, JM101, TG1等；对*endA*-来说可省略，如Top 10和DH5a等，请参照说明书第3页表2。

8. 向离心柱中加入**650 μL DNA Wash Buffer**（确保已加入无水乙醇），室温下，13,000 rpm 离心1分钟，倒掉收集管中的废液，将离心柱重新放回到收集管中。重复步骤"**8**"。

9. 将离心柱放回高速离心机中，13,000 rpm室温下开盖离心2分钟，以彻底去除残留的乙醇。

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。

10. 将离心柱转至一个新的1.5 mL离心管中，向DNA柱的正中间加入**100~150 μL（体积>100 μL）**的**ddH$_2$O（pH在7.0-8.5之间）或Elution Buffer**，室温放置2分钟，13,000 rpm 离心1分钟，洗脱质粒DNA。将洗脱液再加到柱的中间，13,000 rpm 离心1分钟，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，原代细胞及用于微注射，建议去除内毒素（PD1214）。

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
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 ℃ over night.
Low Yield	Low copy-number plasmid.	Increase culture volume and scale up the volume of buffers according to the instruction 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 solution 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	Trace EtOH not completely removed from column.	Make sure that no ethanol residues remain in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.