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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 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.

This kit is designed for fast and efficient purification of plasmid DNA from 100 to 250 mL of *E. coli* culture. The maxi column has a plasmid DNA binding capacity of 1 mg.

The purified plasmid DNA is ready for 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 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 2 to 3 times. Reference Table 1 for the commonly used plasmids,

Table 1 Commonly used plasmids.

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®	Muted pMB1	300-400	350-450
pBluescript®	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. 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. For purifying plasmid DNA from *endA*+ strains (Table 2), we recommend use product PD1713.

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 medium 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 maxi column has an optimal biomass of 450-550. For example, if the OD_{600} is 2.5, the optimal culture volume should be 200 mL.

Culture Volume: Use a flask or tube 4 times larger 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 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 step.

Important

- 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, and then store at 4^{o}C.
- **Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 50^{o}C to dissolve the precipitates before use.**
- Incubating Buffer C1 at 4 ^{o}C before experiment will decrease the floating precipitates.
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure the availability of centrifuge and vacuum manifold, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately either by centrifugation or vacuum.
- ***Carry out all centrifugations at room temperature.***

Materials supplied by users

- 70% ethanol and 100% ethanol
- High speed centrifuge
- 30 mL high speed centrifuge tubes
- 50 mL tubes

Kit Contents

Catalog#	PD1511-00	PD1511-01	PD1511-02
Preps	2	10	25
ezBind™ Columns	2	10	25
Buffer A1	22 mL	110 mL	270 mL
Buffer B1	22 mL	110 mL	270 mL
Buffer C1	27 mL	135 mL	330 mL
Buffer KB	22 mL	110 mL	270 mL
RNase A (20 mg/mL)	2.2 mg (110 µL)	11 mg (550 µL)	27 mg (1.35 µL)
Elution Buffer	6 mL	30 mL	60 mL
User Manual	1	1	1

Safety Information

- Buffer C1 contains acidic acid, wear gloves and protective eyewear when handling.
- Buffer C1 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 Maxiprep Spin Protocol

1. Inoculate **150- 200 mL** LB containing appropriate antibiotic with 100 µL fresh starter culture. Incubate at 37 ℃ for 14-16 h 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 ℃ for 6-8 h with vigorous shaking (~250 rpm).
   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: Do not use a starter culture that has been stored at 4 ℃.
   Note: Do not grow starter culture directly from glycerol stock.

2. Harvest the bacterial by centrifugation at 5,000 x g for 10 minutes at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium completely.

3. Add **10 mL Buffer A1** (Add RNase A to **Buffer A1** before use) and completely resuspend bacterial pellet by vortexing or pipetting **(Complete resuspension is critical for optimal yields).**

4. Add **10 mL Buffer B1**, mix gently but thoroughly by inverting 5-10 times. If necessary, continue inverting the tube until the solution becomes slightly clear. Incubate at room temperature for 5 minutes to obtain a slightly clear lysate.

   Note: Do not incubate longer than 5 minutes. Over-incubating causes genomic DNA contamination and plasmid damage.

5. Add **2.8 mL Buffer C1**, mix immediately by inverting/shaking the vial for 5 times and sharp hand shaking for 5 times.

   Note: It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mix is required to completely neutralize the solution.

6. Transfer the lysate to a high-speed centrifuge tube, centrifuge at 14,000 x g for 20 minutes at room temperature.

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

7. Carefully transfer the clear supernatant into a 50 mL tube (avoid the floating

precipitates). Add **9.2 mL Buffer C1** and **12 mL 100% ethanol.** Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be centrifuged through the DNA column immediately.

8. Immediately apply **20 mL** of the **lysate/ethonal mixture** to a DNA column with the collection tube. Centrifuge at > 2,500 x 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/ethonal mixture** to the DNA column and centrifuge at .2,500 x g for 1 minutes. Discard the flow-through liquid and put the column back to the collection tube.

9. **Optional:** Add **10 mL Buffer KB** into the spin column, centrifuge at > 2,500 x g 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.

10. Add **10 mL 70% ethanol** into the column, centrifuge at > 2,500 x g for 1 minute. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step 9.

11. Centrifuge the column, **with the lid open**, at > 2,500 x g for 10 minutes to remove the ethanol residues.

    Note: It is critical to removes ethanol residues completely. High centrifuge speed (5,000 g) is suggested to remove the ethanol. The remaining ethanol will inhibit the elution of DNA from the column.

12. Carefully transfer the column into a sterile clean 50 mL tube and add **1-1.5 mL Sterile ddH_2O or Elution Buffer** to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 5,000 x g for 5 minutes.

13. For higher yield, reload the eluate in the 50 mL tube to the center of the column and incubate for 1 minute at room temperature. Elute the DNA again by centrifugation at 5,000 x g for 5 minutes.

    Note: If ddH_2O is used for eluting DNA, make sure the pH is ≥ 7.0.
    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 (PD1514) if the DNA is used

for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

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 800 µL 70% ethanol, centrifuge for 5 min, carefully decant. Air-dry the pellet for 10 min. Resuspend the DNA in Elution Buffer or sterile ddH_2O**.**

The DNA concentration can be determined by a spectrophotometer,

DNA concentration (µg/mL) = OD_{260nm} x 50 x dilution factor.

Purification of Low-Copy-Number Plasmid and 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 **400 mL** for the maxiprep.

2. Use **2 x volumes** of the **Buffer A1, Buffer B1** and **Buffer C1** and **100% ethanol**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **Wash Buffer (70% ethanol)** and **Elution Buffer**.

质粒大提试剂盒简明步骤（PD1511）

（详细内容请参考英文说明书）

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。

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：提前预冷或加到裂解液后在冰上放置5分钟利于减少蛋白漂浮。

准备70%和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Wash Buffer (70% 乙醇)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。切勿直接取冻存在4°C的菌进行培养。

柱结合能力：1 mg

III.操作步骤

1. 取100 µL新鲜的菌液接种到**150-200 mL** (勿超过 200 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，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，例如 TB 或 2×YT，请注意保证 OD_{600} 不超过 3.0。

2. 加入**10 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。

3. 加入 **10 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。

4. 加入**2.8 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。

注：使用前将C1预冷或在加到裂解液后在冰上放1分钟利于减少蛋白漂浮。

5. 将离心管转至高速离心机，在室温下14,000 x g 离心20分钟（若上清中有白色沉淀，可再次离心，）。

注：若无高速离心机，此步也可用注射器替代（可单独购买）。

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。

6. 小心吸取离心后的上清液至50 mL管中 *（避免吸起沉淀）*，加入**9.2 mL Buffer C1** 和**12 mL 100% 乙醇**，立即摇匀，需马上离心过DNA柱。

7. 立即转移**20 mL**裂解液/收集管至带收集管的DNA柱中，室温下> 2,500 x g 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。重复此步直至所有的溶液通过DNA柱。

8. 可选：向DNA柱中加入 **10 mL Buffer KB**, 室温下> 2,500 x g 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，请参照英文说明书第3页表2.

9. 向离心柱中加入**10 mL 70% 乙醇**，室温下>2,500 x g 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“9”。

10. 将离心柱放回高速离心机中，室温下>2,500 x g 开盖离心10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。5,000 g 转速更有利于去除乙醇。乙醇是否去除干净将会影响最后的洗脱效率。

11. 将离心柱转至一个新的50 mL离心管中，向DNA柱膜的正中加入**1-1.5 mL 灭菌 ddH_2O （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50 mL离心管中的洗脱液再加到柱的中间，>2,500 x g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，原代细胞及用于微注射，建议去除内毒素（PD1514）。

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℃ 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. Increase the volume of Buffer A1, B1, C1 and ethanol proportionally with the ratio of 1:1:1.2:1.2.
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 elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.