# Contents

# 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 1200 μg. 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 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 Commomly 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 with a volume at 4 times 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

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 instruction booklet and become familiar with each step.

**Important**

- ☑ RNase A: It is stable for half a year under 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 ℃.
- ☑ **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.
- ☑ Make sure the availability of centrifuge, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation.
- ☑ Centrifuge speed at 5,000 x g is recommended. In case the collection tube doesn't fit in high speed centrifuge rotor, use benchtop centrifuge and spin at 2,500 x g with double centrifugation time.
- ☑ ***Carry out all centrifugations at room temperature.***

**Materials supplied by users**

- ☑ 70% ethanol and 100% ethanol
- ☑ High speed centrifuge or vacuum manifold.
- ☑ 30 mL high speed centrifuge tubes and 50 mL tubes.

# Kit Contents

| Catalog # | PD1514-00 | PD1514-01 | PD1514-02 |
|---|---|---|---|
| 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 N3 | 32 mL | 160 mL | 400 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) |
| EndoClean Buffer | 5 mL | 25 mL | 60 mL |
| Endofree Elution Buffer | 5 mL | 25 mL | 60 mL |
| User Manual | 1 | 1 | 1 |

# Safety Information

- Buffer N3 contains acidic acid, wear gloves and protective eyewear when handling.
- Buffer N3 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™ 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. Grow 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 a starter culture that has been stored at 4 ℃.
   **Note:** Do not grow starter culture directly from glycerol stock.
   **Note:** Do not use more than 200 mL culture or cell mass greater than 550. The buffer volumes need to be scaled up if processing over 200 mL of culture.

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 mL Buffer N3**, mix immediately by sharp hand shaking for 5-10 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 sample to a high speed centrifuge tube and centrifuge at 12,000 x g for 10 min at room temperature.

   **Note:** If high-speed centrifuge is not available, Syringe filter (Supplied in PD1515 or purchase separately from Biomiga) could be used to filtrate the lysate.

7. Transfer the clear lysate to a new high-speed centrifuge tube and add **0.1 volume** of **EndoClean Buffer**, vortex for 5s and incubate on ice for 10 min, 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 ℃), the sample becomes turbid after adding **EndoClean Buffer.** The solution becomes clear after incubating on ice.

8. Incubate the solution at 65 ℃ for 5 minutes. The solution becomes turbid again. And then centrifuge at 12,000 x g for 10 minutes (Alternatively, the sample can be processed in a 15 mL conical tube and centrifuge at 2,500 x g for 15 min) at room temperature (the temperature must be higher than 23 ℃). 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 less than 23 ℃.

   **Note:** If phase partitioning is not observed after centrifugation, stand the column for few minutes. Or add **200 µL Chloroform** (37 ℃), vortex to mix well, and centrifuge again.
   **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 20 EU (Endotoxin)/ µg of DNA. Another extraction is necessary if less than 0.1 EU / µg of DNA is desired by repeating step 7-8.

9. Carefully transfer the clear supernatant into a 50 mL tube (avoid the floating precipitates). Add **10 mL Buffer N3** and **12 mL 100% ethanol.** Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be centrifuged through the DNA column immediately.

10. Immediately apply **20 mL** of the **lysate/ethanol mixture** into a DNA column with the collection tube. Centrifuge at > 2,500 x g for 1 min at room temperature. Remove the column from the tube and discard the flow-through liquid. Process the remaining **lysate/ ethanol mixture,** discard the flow-through liquid and put the column back to the collection tube.

11. **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.

12. Add **10 mL 70% ethanol** into the column, centrifuge at > 2,500 x g for 1 min. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step 12.

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

    **Note:** High centrifuge speed (5,000 x g) is suggested to remove the ethanol. Ethanol residues in the column will affect the elution of DNA.

14. Transfer the column to a clean 50 mL tube and add **0.5-1 mL Endofree Elution Buffer** to the center of the column and incubate for 1 minute at room temperature. 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**: Two elutions give rise to maximum DNA yield.
    **Note:** The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, *in vitro* translation, sequencing, transfection, and microinjection.

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

# B. Removal of Endotoxin *after* Plasmid Purification

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

1. Follow the protocol on Page 6 from Step 1 to 6.

2. Transfer the lysate to a clean 50 mL conical tube and add **10 mL** of **Buffer N3** and **12 mL** of **100% ethanol**, mix well and go to Step 10-14 on page 7.

3. After the plasmid is purified, add **0.1 volume** of **Endofree Elution Buffer** to the plasmid sample in a 2 mL centrifuge 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 20 ℃, 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 10s and incubate the tube on ice for 10 min. 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 x g at room temperature for 10 minutes (the temperature must be higher than 23 ℃).The two phases will not separate if the temperature is less than 23 ℃.

   **Note:** If phase partitioning is not observed after centrifugation, add **200 µL chloroform**, vortex to mix well, Centrifuge at 12,000 x g at room temperature for 10 minutes.

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

   **Note:** DNA Precipitates could be seen on the bottom or wall.

7. Add **1 mL 70% ethanol** and centrifuge at 12,000 x g for 5 min.

8. Carefully decant and air-dry the DNA for 10 to 20 minutes in a hood.

9. Resuspend the DNA with **Endofree Elution Buffer**.

   **Note:** The volume of **Endofree Elution Buffer** could be chosen based on downstream application.
   **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 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 volume** of the high copy number culture. Use up to **400 mL** for the maxiprep.

2. Use **2 x volume** of the **Buffer A1, Buffer B1, Buffer N3** and **100% ethanol**. Additional buffers can be purchased from Biomiga.

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

# 无内毒素质粒大提离心法试剂盒简明步骤（PD1514）

（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，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。

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,N3,100%乙醇，相同体积的70% 乙醇和Endofree Elution Buffer。

**转化菌:** 若为-70oC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

对富含内源核酸酶的宿主菌（endA＋）如HB101, JM101, TG1等，需去核酸酶，请使用产品PD1713。

## III. 操作步骤（在提取质粒过程中去除内毒素）

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菌体充分悬浮。

   **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 mL Buffer N3**, 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。

5. 将离心管转至高速离心机，在**室温下**12,000 x g离心10分钟（若上清中有白色沉淀，可再次离心）。

   **注：**若无高速离心机，可用PD1416中提供的针筒式滤器过滤得到上清，该滤器可向Biomiga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10min后再离心。

6. 定量吸取离心后的上清液至新的50 mL管中（避免吸起沉淀），加入**0.1 volume**的**EndoClean Buffer**，混匀后冰浴10分钟，其间不时摇匀(若**EndoClean Buffer**粘稠难吸，可将枪头剪掉头后再吸取)。

   **注**：加入**EndoClean Buffer**后溶液变红并混浊，冰浴后变清亮。

7. 取出后65°C温浴5分钟，室温下(务必使溶液温度恢复到23°C以上，否则溶液不分层)12,000 x g离心10分钟（也可在2,500 x g离心15分钟)。此时溶液分为两层，上层水相含有质粒，下层红色有机相含有内毒素。

   **注：**若离心后上层水相漂浮部分红色小珠，可静止几分钟，待红色小珠自然沉淀。上层残留的红色液体在洗涤时会被一并洗去，将不影响实验结果。也可在加入经温育后的**200 µL Chloroform** (>37°C)，混匀后再重复步骤7，将有利于分层。

   **注：**此时可去除99%的无内毒素，再重复第6-7步可使内毒素含量低于0.1 EU (Endotoxin)/ µg DNA.

8. 将上层水相转移至一个新的50 mL管中，加入**10 mL**的**Buffer N3**及**12 mL**的**100% ethonal**，用手用力甩5次以混匀，需马上离心过DNA柱。

9. 立即将**20 mL**裂解液/乙醇混合液转移至带收集管的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。将剩余溶液转移至DNA柱中，室温下> 2,500 x g离心5分钟，倒掉收集管中的废液，将离心柱重新放回到收集管中。重复直至所有的溶液通过DNA柱。

10. **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，请参照说明书第3页表2。

11. 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“**11**”。

12. 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，较高的转速(2,500 x g)有利于有效的去除乙醇。乙醇是否去除干净将会影响最后的洗脱效率。

13. 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**0.5-1**

**mL**的**Endofree Elution Buffer**，室温放置1分钟，5,000 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，5,000 x g离心5分钟以洗脱质粒DNA。

**注：**提取到的质粒DNA可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。

# Trouble Shooting Guide

| **Problems** | **Possible Reasons** | **Suggested Improvements** |
|---|---|---|
| Low Yield | Poor Cell lysis. | • Resuspend pellet thoroughly by votexing and pipetting prior adding Buffer B1.<br>• 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 and the volume of Buffer according to 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 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 if necessary. |

***FOR RESEARCH USE ONLY.**