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Introduction

Key to this kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily eluted with sterile water or Elution Buffer.

Plasmid isolated with traditional protocol normally contains high level of endotoxins (lipopolysaccharides or LPS). For transfection of endotoxin sensitive cell lines or microinjection, the endotoxins should be removed before the applications. The Ezgene™ endofree system uses a specially formulated buffer that extracts the endotoxin from the plasmid DNA. Two rounds of extraction will reduce the emdotoxin level to 0.1 EU (Endotoxin) per μg of plasmid DNA. The endofree plasmid miniprep kit provides an efficient endotoxin removal step into the traditional purification procedure to produce transfection grade plasmid DNA

This kit is designed for fast and efficient purification of plasmid DNA from 3 to 12 mL of *E. coli* culture. The miniprep column has a plasmid DNA binding capacity of 80 μg.

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

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 times. Please contact our customer service for further information and reference the Table 1 for the commonly used plasmids,

Table 1 Commonly used plasmids.

Plasmid	Origin	Copy Numbers	Expected Yield (μg per 10 mL)
pSC101	pSC101	5	0.5-0.75
pACYC	P15A	10-12	1-1.5
pSuperCos	pMB1	10-20	1-2.5
pBR322	pMB1	15-20	1.5-2.5
pGEM®	Muted pMB1	300-400	30-40
pBluescript®	ColE1	300-500	30-50
pUC	Muted pMB1	500-700	50-70

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. Please reference Table 2 for the *endA* information.

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 mini column has an optimal biomass of 30-45. For example, if the OD_{600} is 3.0, the optimal culture volume should be 10-15 mL.

Culture Volume: Use a flask or tube 4 times bigger in volumn 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

Alternative endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA, while 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 steps and pay special attention to the followings.

Important

- RNase A: It is stable for half of year under room temperature. Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use.
- Add 8 mL (PD1212-00) or 60 mL (PD1212-01) or 96 mL (PD1212-02) 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 and 2.0 mL pyrogen free microcentrifuge tubes
- High speed microcentrifuge
- Vacuum manifold if vacuum protocol is applied.

Kit Contents

Catalog #	PD1212-00	PD1212-01	PD1212-02
Preps	4	50	250
ezBind Columns	4	50	250
Buffer A1	2.5 mL	25 mL	125 mL
Buffer B1	2.5 mL	25 mL	125 mL
Buffer N3	4 mL	30 mL	175 mL
Buffer KB	3 mL	30 mL	135 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL
EndoClean Buffer	1 mL	10 mL	40 mL
Endofree Eluiton Buffer	1 mL	10 mL	30 mL
RNase A (20 mg/mL)	0.25 mg (12.5 µL)	2.5 mg (125µL)	12.5 mg (625 µL)
User Manual	1	1	1

*Add 8 mL (PD1212-00) or 60 mL (PD1212-01) or 96 mL (PD1212-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

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 Miniprep Protocol A:
Removal of Endotoxin *during* Plasmid Purification

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

A1. Spin Protocol

1. Inoculate **3-12 mL** LB containing appropriate antibiotic with a fresh colony. Grow at 37 ℃ for 14-16 hours with vigorous shaking.

   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 (OD_{600}). Buffers needs to be scaled up if over amount of cultures are being processed.
   Note: Do not use a starter culture that has been stored at 4 ℃.
   Note: Do not grow starter culture directly from glycerol stock.
   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.

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

3. Add **450 µL Buffer A1** and completely resuspend bacterial pellet by vortexing or pipetting **(Complete resuspension is critical for optimal yields).**

4. Add **450 µL Buffer B1**, mix gently by inverting 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 50 ℃ to dissolve precipitation before use.

5. Add **100 µL Buffer N3**, mix completely by inverting/shaking the vial for 5 times and sharp hand shaking for 2 times.

   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 12,000 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 the **clear lysate** into 2.0 mL tube and add **0.1 volume** of **EndoClean Buffer.** Mix by vortexing till homogeneous and incubate on ice for 10 minutes. Mix by tapping the tube several times during incubation.

   Note: The solution becomes red and turbid after adding EndoClean Buffer. The solution becomes clear after incubation on 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.

8. Centrifuge the solution at 12,000 rpm for 10 minutes at room temperature (the temperature must be greater 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:
   - Incubate the solution at 65℃ for 5 minutes. The solution becomes turbid again. And then repeat step 8.
   - Or add 200 µL Chloroform (37℃), vortex to mix well, repeat step 8**.**

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

9. Transfer the clear phase lysate, avoid the colored phase, to a clean 2.0 mL. Add **450 µL** of **Buffer N3** and **400 µL** of **100% ethonal**. Mix well by sharp hand shaking for 3 times.

10. Transfer **700 µL** of the **lysate/ethanol mixture** to a DNA column and centrifuge at 13,000 rpm for 20s. Decant the flow-through liquid and insert the column back to the collection tube. Transfer the **remaining solution** to the column and centrifuge at 13,000 rpm for 20s. Decant the flow-through liquid and insert the column back to the collection tube.

11. **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 for details.

12. Add **650 µL** of **DNA Wash Buffer** and centrifuge at 13,000 rpm for 20s. Decant the flow-through liquid and insert the column back to the collection tube. Repeat step "12".

13. Centrifuge the column, **with the lid open**, at 13,000 rpm for 2 minute.

    Note: It is critical to removes ethanol residues completely. The remaining ethanol will inhibit the elution of DNA from the column.

14. Transfer the column to an endofree 1.5 mL tube and add **50-100 µL** of **ddH_2O** or **Endofree Elution Buffer（Supplied）**. Incubate for 1 minute and centrifuge at 13,000 rpm for 1 minute to elute DNA. Reload the eluate into the column (use the same 1.5 mL tube) and incubate for 1 minute, centrifuge at 13,000 rpm for 1 minute to elute DNA.

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

15. Determination of DNA concentration,
    Concentration (µg/mL) = $OD_{260\ nm}$ x 50 x dilution factor.

    Note: Two elutions give rise to maximum DNA yield. Use less **EndoFree Elution Buffer** if high concentration is desired.

A2. 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 1-9 in previous protocol on page 6 and 7.

3. Carefully transfer the clear lysate from step 9 in the previous protocol to a 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 buffer pass 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.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. Transfer the column to an endofree 1.5 mL tube and add **50-100 µL** of **ddH_2O** or **Endofree Elution Buffer（Supplied）.** Incubate for 1 minute and centrifuge at 13,000 rpm for 1 minute to elute DNA. Reload the eluate into the column (use the same 1.5 mL tube) and incubate for 1 minute, centrifuge at 13,000 rpm for 1 minute to elute DNA.

   Note: The DNA is ready for transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

EZgene ™ EndoFree Plasmid Miniprep Protocol 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. Carefully transfer the clear lysate to a 1.5 mL tube and add **500 µL Buffer N3** and **400 µL 100% ethanol**. Mix well by sharp hand shaking for 2 times and go to step 10-15 on page 9-10.

   Note: The plasmid DNA is purified. The following steps are for removal of endotoxin.

3. After the plasmid is purified, add **0.1 volume** of **EndoClean Buffer** to the plasmid sample in a 2 mL centrifuge tube (For example, add **10 µL EndoClean Buffer** to **100 µL plasmid sample**). The solution becomes turbid after adding EndoClean Buffer. If the temperature is below 20 ℃, the solution remains clear.

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. Centrifuge at 12,000 rpm at room temperature for 10 minutes **(the temperature must be greater than 23 ℃ for phase partitioning).** Carefully transfer the upper clear layer solution to another 2 mL tube.

   Note: If phase partitioning is not observed after centrifugation, add **200 µL Chloroform**, votex for 10s, and repeat step5.

6. Precipitate plasmid DNA with **0.1 volume** of **3 M KAc (pH 5.2)** and **0.7 volume of Isopropanol.** Centrifuge at 12,000 rpm for 10 min. Carefully decant the supernatant.

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

8. Carefully decant and air-dry the DNA for 30 minutes in a hood. Resuspend the DNA with **ddH_2O** or **Endofree Elution Buffer（Supplied）.**.

   Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, in vitro translation, sequencing, transfection, and microinjection.

无内毒质粒小提试剂盒简明步骤（PD1212）

（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。使用前请将提供的所有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 (PD1212-00)或60 mL (PD1212-01)或96 mL (PD1212-02) 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，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,N3,相同体积的Wash 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。

切勿直接取冻存的菌种进行培养。

III. 操作步骤

1. 接种新鲜的单个菌落到**3-12 mL**的LB培养基 (含适量抗生素)，37°C震荡培养14-16小时。

   注：残留的液体培养基容易导致菌液裂解不充分，第5步离心后沉淀较松，不能有效吸取上清。

   注：本操作程序适用于标准 LB (Luria Bertani) 培养基培养 12-16 小时，OD_{600} (细菌密度) 在 2.0-3.0 之间的菌液。若用的是富集培养基，例如 TB 或 2×YT，请注意保证 OD_{600} 不超过 3.0。

2. 室温下10,000 x g离心1分钟，收集菌体，并尽可能的吸去上清。
3. 加入**450 µL 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。

4. 加入 **450 µL 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**100 µL 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。

6. 将离心管转至高速离心机，在**室温下**13,000 rpm离心10分钟*(若上清中仍有白色沉淀，可翻转后再次离心5分钟)*。

7. 定量吸取离心后的上清液至新的1.5mL管中，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。

8. 室温下*(冰浴取出后务必使溶液温度恢复到23°C以上，否则溶液不分层，为保证分层效果，可直接在65°C温育5分钟)*13,000 rpm离心10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无内毒素。

   注：若分层不明显，则是因为温度不够，可65°C温浴5min，再在13,000 rpm离心10分钟。或者加入经温育后的**200 µL Chloroform** (>37°C)，再重复步骤8.

   注：此时可去除99%的无内毒，再重复第7-8步可使内毒素含量低于0.1 EU (Endotoxin)/ µg of DNA.

9. 将上层水相转移至一个新的2.0 mL管中，加入**450 µL**的**Buffer N3**及**400 µL**的**100% ethonal**，用手用力甩3次以混匀。

10. 将溶液**700 µL**转移至带有收集管的DNA柱中，室温下13,000 rpm 离心20秒，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

步骤至全部溶液转移至DNA柱中。

11. **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页的表2。

12. 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“12”。

13. 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。

14. 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**50~100 µL (体积>50 µL)** 的**Endofree Elution Buffer**，洗脱质粒DNA。将离心管中的洗脱液再次加到DNA柱的正中间，室温放置1分钟，13,000 rpm 离心1分钟，收集洗脱液到同一个离心管中。

    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。

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 up to 25 mL for miniprep II.

2. Use **2 x volumes** of the **Buffer A1, Buffer B1, Buffer N3, and Buffer KB**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **DNA Wash Buffer** and **Endofree 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 Buffer N3, and Buffer KB**. Additional buffers can be purchased from Biomiga.

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

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

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 according to instructions on page 14.
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.

Related Products

Catalog #	Product Name	Preps	Price $
PD1211-01	Plasmid mini kit	50	45.00
PD1211-02	Plasmid mini kit	250	220.00
PD1213-01	Plasmid mini kit II	50	60.00
PD1213-02	Plasmid mini kit II	250	250.00
PD1411-01	Plasmid midi kit	10	52.00
PD1411-02	Plasmid midi kit	25	125.00
PD1412-01	Plasmid midi kit II	10	79.00
PD1412-02	Plasmid midi kit II	25	175.00
PD1413-01	Plasmid ezFilter midi kit	10	79.00
PD1413-02	Plasmid ezFilter midi kit	25	175.00
PD1511-01	Plasmid maxi kit	10	120.00
PD1511-02	Plasmid maxi kit	25	250.00
PD1512-01	Plasmid ezFilter maxi kit	10	130.00
PD1512-02	Plasmid ezFilter maxi kit	25	280.00
PD1611-01	Plasmid ezFilter mega 3 kit	2	80.00
PD1611-02	Plasmid ezFilter mega 3 kit	10	380.00
PD1811-01	96-well plasmid mini kit	4x96	400.00
PD1811-02	96-well plasmid mini kit	20x96	2000.00