Ver: 2404

EZgene™ EndoFree Plasmid ezFilter Maxiprep Kit (BW-PD1515)

Contents

BW-PD1515 EZgene™ EndoFree Plasmid ezFilter Maxiprep Kit

Kit Contents

Catalog#	BW-PD1515-00	BW-PD1515-01	BW-PD1515-02
Preps	2	10	25
Maxi Columns	2	10	25
50 mL Collection Tubes	2	10	25
Filter syringe (60 mL)	2	10	25
Buffer GBL	8 mL	30 mL	70 mL
Buffer A1	22 mL	110 mL	270 mL
Buffer B1	22 mL	110 mL	270 mL
Buffer N3	28mL	140mL	330mL
EndoClean Buffer	5 mL	25mL	60 mL
DNA Wash Buffer*	15 mL	54 mL	2x54 mL
EndoFree Elution Buffer	5 mL	25 mL	60 mL
RNase A (20 mg/mL)	110 µL	550 µL	1.35 mL
User Manual	1	1	1

*Add 60 mL (BW-PD1515-00) or 216 mL (BW-PD1515-01) or 216 mL (BW-PD1515-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

Key to the plasmid purification kit is our proprietary DNA binding system that allows the highly 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 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 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. 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 Maxi Column has an optimal biomass of 400-500. For example, if the OD_{600} is 2.5, the optimal culture volume should be 150 to 200 mL. For over amount of cell numbers, either reduce the biomass or scale up the volume of Buffer A1, B1 and N3.

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 2-8℃ 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: 20 mg/mL. It is stable for one year at room temperature (15-25℃). Spin down RNase A vial briefly before adding to Buffer A1.

✪ Buffer A1 should be stored at 4℃ once RNase A is added.

✪ Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 37℃ to dissolve the precipitates before use. Keep the cap tightly closed for Buffer B1 after use.

✪ Buffer N3 may form precipitates upon storage, warm up at 37℃ to dissolve the precipitates before use.

- Ensure the availability of centrifuge capable of 8,000 ×g.
- *Carry out all centrifugations at room temperature.*

Materials not Supplied

- High speed centrifuge.
- 96-100% ethanol.
- 50 mL centrifugal tubes.

Safety Information

Buffer N3 contains acidic acid, wear gloves and protective eyewear when handling.

EZgeneTM 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. 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 yield may be reduced.

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

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

   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: This protocol is optimized for *E. coli* strain cultured in LB medium. When using TB or 2 × YT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD_{600}). Buffers need to be scaled up proportionally if over amount of cultures are being processed.

2. Column equilibration: Place a **Maxi Column** in a clean collection tube, and add **2.5 mL Buffer GBL** to **Maxi Column**. Centrifuge for 2 min at 8,000 rpm in a table-top centrifuge. Discard the flow-through, and set the **Maxi 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 **10 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 **9 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℃ to dissolve the precipitations before use.

6. Add **2 mL Buffer N3**, 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: If the amount of RNA in the bacterial solution is large, it can be allowed to stand for 10 minutes to make the RNase fully function.

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 8,000 ×g for 10-15 minutes at room temperature. Transfer the cleared lysate to a 50 mL centrifugal 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 centrifugal 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 centrifugal 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. 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°C), the sample becomes turbid after adding EndoClean Buffer. The solution becomes clear after incubating on ice.

9. Incubate the solution at 65°C for 5 minutes. The solution becomes turbid again. And then centrifuge at 8,000 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°C). 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 °C.

   Note: If phase partitioning is not observed after centrifugation, stand the column for few minutes. Or add 200 µL Chloroform (37°C), 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 step8-9.

10. Carefully transfer the clear lysate into a 50 mL centrifugal tube, (avoid the floatingprecipitates). 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.

11. Immediately apply **20 mL** of the lysate/ethanol mixture to a **pretreated Maxi Column** with the **50 mL Collection Tube**. Centrifuge at 8,000 ×g for 1 minute at room temperature. Discard the flow-through liquid and put the column back to the **50 mL Collection Tube**. Add the remaining lysate/ethanol mixture to the DNA column and centrifuge at 8,000 ×g for 1 minute. Discard the flow-through liquid and put the column back to the collection tube.

Note: The Maxi column has a maximum capacity of 20 mL. If apply 20 mL of the lysate/ethanol mixture to the Maxi Column, you should incubate 2-5 minutes at room temperature (avoid splashing of the mixture during centrifugation).

12. Add **10 mL DNA Wash Buffer** (*Add ethanol to DNA Wash Buffer before use*) into the **Maxi Column**, centrifuge at 8,000 ×g for 1 minute, discard the flow-through.

13. Add **3 mL 100% ethanol** into the column, centrifuge at 8,000 ×g for 1 minute, discard the flow-through.

14. Reinsert the column, with the lid open, into the 50 mL centrifugal tube and centrifuge for 10 minutes at 10,000 ×g.

Note: It is critical to remove residual ethanol completely. Residual ethanol can be removed more efficiently with 50-60℃ for 10 minutes after centrifuge.

15. Carefully transfer the **Maxi Column** into a sterile 50 mL centrifugal tube and add **1.5-2 mL** sterile ddH_2O or **EndoFree Elution Buffer (preheating at 65℃)** into the center of the column and let it stand for 1 minute. Elute the DNA by centrifugation at 10,000 ×g for 5 minutes.

Optional: Reload the eluate into the center of the column for a second elution.

Note: The first elution normally yields around 70% of the plasmid DNA. Add the eluted DNA back to the column for another elution yields 20-30% of the DNA.

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 if the DNA is used for endotoxin-sensitive

cell lines, primary cultured cells or microinjection.

16. The DNA concentration can be calculated as follows,

Concentration (μg/mL)=OD_{260}×50×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 6-7 from Step 1 to7.

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 11-15 on page 8.

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 **0.1 mL EndoClean Buffer** to **1 mL plasmid sample**).

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°C 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°C). 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 °C.

   Note: If phase partitioning is not observed after centrifugation, stand the column for few minutes. Or add 200 μL Chloroform (37°C), vortex to mix well, and centrifuge again.

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. Carefully decant and air-dry the DNA for 30 minutes in a hood.

8. 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/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 **400 mL** for the maxiprep.

✪Use **2×volumes** of the **Buffer A1, Buffer B1, Buffer N3**.

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

无内毒素质粒大提离心法简明步骤（PD1515）

（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，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 N3：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,N3,100%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：1mg

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PD1712。

III. 操作步骤 （在提取质粒过程中去除内毒素）

1. 取50 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，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**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。

4. 加入 **9 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**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。

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15 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15 mL的试管上静置10分钟。管中的白色絮状沉淀浮上来，对准15 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. 定量吸取离心后的上清液至新的15 mL管中（避免吸起沉淀），加入**0.1 倍体积**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：静置10 分钟时RNase A将工作，排除RNA污染。若离心十分钟后再用过滤器过滤更有利于去除蛋白。

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

8. 取出后65℃温浴5min，室温下(务必使溶液温度恢复到23℃以上，否则溶液不分层)8,000 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℃)，混匀后再离心10分钟。

   注：此时可去除99%的无内毒素，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，需马上离心过预处理DNA柱。

10. 立即转移**20 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。

11. 向吸附柱中加入**10 mL DNA Washing Buffer**，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。重复步骤“**11**”。

12. 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，将吸附柱重新放回到收集管中。
13. 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，将吸附柱在65度烘箱中放置10分钟有助于彻底去除乙醇， 提高 **Endofree Elution Buffer** 的洗脱效率。

IV. 操作步骤 （在提取质粒后去除内毒素）

该步骤用于质粒纯化后内毒素去除。

1. 按照 III 操作步骤中 1-6 步进行（第 10-11 页），转移上清至新的 50 mL 离心管中。加入 **10 mL Buffer N3** 和 **12 mL 100%乙醇**。混匀。之后按照 III 操作步骤中的 11-14 步继续实验操作，得到质粒。
2. 加入 **0.1 倍体积**的 **EndoClean Buffer** 至含质粒样品的 2.0 mL 无菌高速离心管（例如，加入 **0.1 mL EndoClean Buffer** 至 1 mL 质粒样品）。
3. 涡旋几次，冰上静置 10 min，直至溶液澄清不浑浊。（若可行的话，建议将样品置于冷室震荡 10 min）颠倒混匀。
4. 室温 12,000 ×g 离心 10 min。若离心温度低于 23℃，将不会见到分层现象。
   注：若离心后没有见到分层现象，65℃孵育 5 min，溶液又变得浑浊，重复步骤 4。或者加入 200μL 体积的氯仿（37℃），涡旋混匀 10 s，室温 12,000×g 离心 5min。
5. 小心转移上层裂解液至另一个高速离心管内。
6. 加入 **0.1 倍体积**的 **3 M KAc（pH5.2）**或 **NaAc（pH5.2）**和 **0.7 倍体积**的**异丙醇**，沉淀质粒 DNA。
7. 12,000 ×g 离心 10 min，小心弃上清。
8. 加入 **2 mL 70%乙醇**，12,000 ×g 离心 5min，小心弃上清，室温干燥 10-20min。
9. 加入 **EndoFree Elution Buffer**，重悬质粒 DNA。
   注：纯化后的 DNA 可用于内毒素敏感细胞系、原代细胞的转染和显微注射。

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low yield	Poor cell lysis.	Resuspend pellet throughly by vortexing and pipetting prior to adding Buffer B1.
		Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1 : 0.2 M 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 overnight.
Low yield	Low copy number plasmid.	Increase culture volume and the volume of Buffer A1, B1,

		N3 as instructed on page 10.
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	Ethanol traces were not completely removed from column.	Make sure that no ethanol residue remains in the silicon membrane before elute 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, express 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.

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